

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**Effects of orally administered ovine
serum immunoglobulin in the normal
and *Salmonella enteritidis* – challenged
growing rat.**

A thesis presented in partial fulfilment of the requirements for
the degree of
Doctor of Philosophy in Nutrition at Massey University,
Palmerston North, New Zealand

Prabhu Balan
2011



MASSEY UNIVERSITY

*I am dedicating my thesis to
my guru, his holiness
arulthiru BANGARU
ADIGALAR*

Abstract

Immunoglobulins (Ig) are the primary anti-infective component of plasma, colostrum and breast milk. They are the specialized glycoproteins that protect the body from harmful bacteria, viruses and other environmental pathogens by either binding to them or by forming an encapsulating barrier. The development of antimicrobial and immunomodulatory products from natural sources for dietary supplementation in both animals and humans is an active area of research. Purified Ig from sheep plasma (ovine serum Ig) is one such candidate product.

Based on the results of the numerous background growth studies of others, the objectives of this study were to determine whether orally administered ovine serum Ig affected growth performance, digestive organ weights, gut morphology, immunity, the gut microbiota, goblet cell numbers, mucin gene expression and digesta mucin protein contents in the growing rat. The study also sought to understand whether orally administered ovine serum Ig prevented or lessened the negative effects of *Salmonella enteritidis* ATCC 13076 (a pathogen) in the *S. enteritidis*-challenged growing rat. The presence of ingested intact Ig in different parts of the digestive tract was also determined. Investigations were undertaken in normal and *S. enteritidis*-challenged Sprague-Dawley male growing rats. Diets were iso-caloric and had similar protein and amino acid contents. The diets were fed for 21 days (for non-challenged rats) and for 18 days (for the challenged rats).

An ovine Ig fraction improved food conversion efficiency, the weights of several digestive organs and gut histology. Compared with spray-drying, a freeze-drying procedure preserved a higher degree of immunological activity.

In immunity studies, an ovine Ig fraction selectively enhanced ($P < 0.05$) various indices of immune function such as phagocytic activity, lymphocyte proliferation and gut and plasma antibodies. In microbiological studies, the number of lactobacilli in the gut were increased ($P < 0.05$) by feeding the ovine Ig. Ovine Ig also influenced the transcription and translation of gut mucin protein as evidenced by increased ($P < 0.05$) mucin gene expression and digesta mucin protein concentrations as well as an increased goblet cell count.

After gavaging with *S. enteritidis*, the rats fed the IOI (inactivated ovine Ig) and BD (basal diet) diets grew considerably more slowly (growth declined)

than the challenged rats fed the FDOI (freeze-dried ovine Ig) diet and the latter rats showed no sign of infection. The villus length, crypt depth, villus:crypt ratio and villus surface area (VSA) of the duodenum and jejunum were generally greater ($P < 0.05$) in rats challenged with *S. enteritidis* and receiving the FDOI diet compared to either the unchallenged rats fed the BD diet (except duodenal and jejunal VSA) or the challenged rats fed the BD or IOI diets. Several measures of immune modulation were affected as was the bacterial composition of the gut microflora. The ileal and colonic digesta for the FDOI-fed rats had higher ($P < 0.05$) numbers of goblet cells and higher ($P < 0.05$) digestive luminal mucin protein concentrations than the challenged rats fed either the BD- or IOI-supplemented diets.

Intact ovine Ig were detected in the luminal contents from the stomach through to the colon in the growing rat fed orally with ovine Ig fraction. The amounts (percentages of digesta dry matter) of intact ovine Ig for rats fed the FDOI diet were 2.17%, 3.12%, 5.31%, 2.03% and 5.76% for stomach chyme, duodenal, jejunal, ileal and colonic digesta respectively. Overall, the accumulated amount was 18.4%, which indicates the presence of a high level of active material throughout the digestive tract.

In conclusion, purified ovine Ig improves growth of healthy rats and protects against enteric infection by immunomodulation, mucin protein and/or modification of commensal microbial composition. The results contribute to knowledge of how orally administered ovine Ig can modulate and enhance key indicators of gut function and overall growth performance in the growing rat.

ACKNOWLEDGEMENTS

PhD is not just a degree, it's a discovery journey. I didn't do this journey alone. So, there are many kind hearted well-wishers I need to acknowledge and thank.

First and foremost my chief supervisor Distinguished Professor Paul J Moughan for his kind support, motivation, immense patience, deep scientific insights, dedication and willingness to let me explore unusual avenues in order to solve research problems and satisfy my curiosity. Without his scholarly insight, substantial corrective comments, prompt and high-quality feedback, my PhD thesis would not have appeared in its present form.

I am thankful to my second supervisor Professor Harjinder Singh for his kind encouragement, guidance and motivation for my PhD journey. I also take this opportunity to especially thank my third supervisor, Dr Kyoung-Sik Han; without his continuous help, supervision and advice I would not have completed my PhD journey. I would like to thank Dr Shane M Rutherford for his invaluable support, scientific advice and guidance for my research work. Without his reference letter, I would not have received a hardship bursary.

I express my deep sense of gratitude to Dr Kay Rutherford-Markwick for her immense support in completing the immunology research works and publications. I should also like to thank Dr Mike Boland (Riddet Institute), Prof Gerald Tannock and Dr Blair Lawley (Department of Microbiology and Immunology, University of Otago) and Dr Nicole Roy (Food Nutrition and Genomics, AgResearch Ltd) for their valuable comments in the preparation of the thesis chapter and manuscript describing the DGGE work.

I am thankful to Dr Venkata Sayoji Rao Dukkupati (Institute of Veterinary, Animal and Biomedical Sciences, Massey University) for his kind assistance in performing Western Blot studies. I also thank Dr Ganesalingam (Institute of Fundamental Sciences, Massey University) for his valuable advice on the statistical analysis.

I am deeply grateful to Professor Ravi Ravindran and Dr Rana Ravindran for their valuable support during PhD.

I am truly thankful to the Riddet Institute for providing me PhD Riddet Scholarship, Research Assistant position during my 4th year of PhD and now Research Officer Position. I am also thankful to Massey University for providing me with a Bailey Bequest Bursary.

I am indeed thankful to Ms Willi Twight, Ms Felicia Stibbards, Ms Ansley Te Hiwi, Ms Paula McCool, Mr John Henley-king, Mr Mark Ward and Mr Andy Lim and Ms Terri Palmer for their immense cooperation, administrative assistance and invaluable support.

I am very grateful to Mr Jack Cui for his kind support in preparing the sheep immunoglobulin fraction.

I am extremely thankful to Mr Joe Crenshaw and Mr Scott Dorr (American Protein Corporation, APC, USA) for providing me with a free sample of spray dried porcine plasma - AP920 for my research work.

I appreciate everyone at the Riddet Institute who played the role of counsellors as well as well-wishers. Especially I would like to thank Mr Shantanu Das, Ms Sharon Henare, Ms Janiene Gilliland, Mr Carlos Montoya, Mr Mallesh Peram, Mr Anant Dev, Ms Lovedeep Kaur, Mr Arup Nag, Ms Anwesha Sarkar, Ms Maggie Zou and Mr Guillaume Brisson. I am also thankful to all based at the Institute of Food Nutrition and Human Health for their support. A special thanks to Ms Kelly O'Flaherty and Ms Shay Rutherford for their kind support during my animal trials. Special thanks to Mr Mathew Levin for his IT support.

I take this opportunity to say a big thanks to Ms Michelle McGrath, Ms Anne Broomfield and Ms Shampa de (Institute of Food Nutrition and Human Health) for their full hearted support in the immune work and DGGE study.

I am deeply grateful to Ms Debbie Chesterfield (Manager, Small Animal Plant Unit, Massey University, Palmerston North) for her enormous contribution during animal studies.

I would not have completed my animal trials without approval from the Animal

Ethics Committee, Massey University, thank you. I would like to extend my heartfelt thanks to Ms Juliet Cayzer (Estendart, Palmerston North) for her guidance during my *S. enteritidis* – challenged rat trial.

I wish to sincerely thank Mr. Aidan Wood, IT Assistant, Massey University, for his valuable support in making my table of contents in PhD thesis.

The blessings of my family have given me the strength to complete this PhD research study to the best of my effort. There are no words to explain the affection and love that I have for my family. I am not here, without my parents Mr Sambasivam Balan Mudliyar and Ms Punithavathi Balan (late) and Ms Selvi Balan and I thank them for their constant love, encouragement and guidance. I am extremely thankful to my eldest sister Ms Saraswathi Shankar, my second eldest sister Malleshwari Suresh and younger sweet and lovely sister Ms Yamini Balan for their constant prayers, motivation and love. I am thankful to my brothers-in-law, Mr. Shankar, Mr. Suresh, Mr Karthick. Last but not least, my final thanks and undying gratitude go to my lovely wife and kids, Ms Suchitra Prabhu, Master Sakthi Bala Prabhu and Master Kavin Sakthi Prabhu. Susi, Yamini, Sakthi, and kutty Sakthi without your love, endurance, strength, prayer and patience, I would not have completed this PhD journey. Thank you.

TABLE OF CONTENTS

Abstract	i
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	vi
LIST OF TABLES	xvi
LIST OF FIGURES	xix
LIST OF ABBREVIATIONS	xxi
LIST OF PUBLICATIONS	xxvi
Chapter 1	1
Review of Literature.....	1
1.1. The gut: its structure and function	2
1.1.1. Tight junctions (TJ)	3
1.1.1.1. TJ structure and composition	3
1.1.1.2. Permeability through TJ	4
1.1.1.3. Factors affecting TJ.....	5
1.1.1.3.1. Food.....	5
1.1.1.3.2. Microbes.....	6
1.1.1.3.3. Diseases	7
1.1.2. M-cells	7
1.1.3. Paneth cells	7
1.1.4. Peyer's patches.....	8
1.1.5. Lamina propria	8
1.1.6. Dendritic cells (DC)	8
1.1.7. Gut enzymes and secretions.....	9
1.2. The gut's role in host health.....	11

1.2.1. The gut barrier.....	12
1.3. Gut microbiota.....	13
1.3.1. Commensal bacteria.....	16
1.3.2. Probiotics	16
1.3.3. Prebiotics	17
1.3.4. Synbiotics.....	17
1.4. Gut mucosal layer	18
1.4.1. Diet effects on mucin	21
1.4.2. Mucus synthesis and secretion: the effect of microbial derived factors	22
1.4.3. Microbial mucolysis	23
1.5. Gut Immune System.....	24
1.5.1. Innate immune system	25
1.5.1.1. Sensor Molecules	26
1.5.1.1.1. Toll-like receptors.....	27
1.5.1.1.1.1. RIG-I–like receptors	29
1.5.1.1.1.2. Nod-like receptors (NLR).....	29
1.5.1.1.1.3. C-type lectin receptor (CLR).....	30
1.5.1.2. Effector Molecules	30
1.5.1.2.1. Hydrogen peroxide and nitric oxide	30
1.5.1.2.2. Defensins.....	31
1.5.1.2.2.1. α -defensins	31
1.5.1.2.2.2. β -defensins	32
1.5.1.2.3. Hepcidins	33
1.5.1.2.4. Cathelicidins	33
1.5.1.2.5. Lysozyme.....	34
1.5.1.2.6. Secreted Phospholipase A ₂ (PLA ₂)	34
1.5.1.2.7. Lectins	35
1.5.1.2.8. Other proteins	35

1.5.2. Adaptive immune system	36
1.6. Host–pathogen interactions	39
1.6.1. Breakdown of gut barrier function.....	41
1.6.2. Translocation of pathogens	41
1.6.3. Microbial toxins	42
1.6.3.1. cAMP	43
1.6.3.2. cGMP	43
1.6.3.3. Calcium signalling	44
1.6.3.4. Nitric oxide	44
1.6.3.5. Pore forming toxins	44
1.6.3.6. Toxins blocking and inducing protein synthesis	44
1.6.3.7. Toxins influencing the enterocyte actin cytoskeleton	45
1.7. Immunoglobulins.....	45
1.7.1. Functions of Ig	45
1.7.2. Structure of Ig	45
1.7.2.1. Classes and types of Ig	47
1.7.2.1.1. Ig classes	47
1.7.2.1.2. Ig Subclasses	48
1.7.2.1.3. Ig Types	48
1.7.3. Ig: Mechanism of action	48
1.7.3.1. Opsonisation	48
1.7.3.2. Activation of complement	49
1.7.3.3. Antibody Dependent Cell-mediated Cytotoxicity (ADCC)	49
1.7.3.4. Intestinal Ig – Immune exclusion and inclusion.....	49
1.7.4. Antibody-mediated immunity	51
1.7.5. Digestion of Ig by digestive enzymes	52
1.7.6. Ig recovery	54
1.7.7. Sources of Ig.....	54

1.7.7.1. Monoclonal antibodies	54
1.7.7.2. Egg yolk antibodies (IgY)	55
1.7.7.3. Colostrum and milk Ig	55
1.7.7.4. Animal plasma	56
1.7.7.5. Plasma Ig	60
1.7.8. Ig as a dietary supplement.....	61
1.8. Conclusion	64
1.9. Literature cited	67
Chapter 2.....	105
Orally administered ovine serum immunoglobulins influence growth performance, organ weights and gut morphology in growing rats.....	105
2.1. Abstract.....	106
2.2. Introduction	107
2.3. Materials and methods	108
2.3.1. Preparation and ELISA of ovine serum Ig and SDPP	108
2.3.2. Animal study.....	109
2.3.3. Chemical analysis.....	110
2.3.4. Growth performance.....	110
2.3.5. Post-mortem procedure and organ weights	111
2.3.6. Gut morphology.....	111
2.3.1. Statistical analysis	113
2.4. Results	113
2.4.1. Biological activity of freeze-dried ovine Ig	113
2.4.1.1. Growth performance	113
2.4.1.2. Organ weights	114
2.4.1.1. Gut morphology	114
2.4.2. Comparison between freeze- and spray-dried ovine Ig.....	115
2.4.3. Growth performance.....	115
2.4.3.1. Organ weights	115

2.4.3.2. Gut morphology.....	116
2.5. Discussion	116
2.6. Literature cited	122
Chapter 3.....	127
Immunomodulatory effects of ovine serum immunoglobulin in the growing rat.....	127
3.1. Abstract	128
3.2. Implications.....	129
3.3. Introduction.....	129
3.4. Material and methods	130
3.4.1. Preparation and quantitation of ovine serum Ig	130
3.4.2. Animal study	131
3.4.3. Chemical analysis	131
3.4.4. Post-mortem procedure	133
3.4.5. Assessment of phagocytosis.....	133
3.4.6. Preparation of splenocytes.....	134
3.4.6.1. Lymphocyte proliferation assay	134
3.4.7. Analysis of cytokines.....	135
3.4.8. Quantitative analysis of IgA and IgG	135
3.4.9. Statistical analysis.....	136
3.5. Results.....	136
3.5.1. PBL phagocytosis	136
3.5.2. Lymphocyte proliferation	137
3.5.3. Cytokine analysis	137
3.5.1. Rat IgA and IgG concentrations in intestinal digesta and plasma	137
3.6. Discussion	138
3.7. Literature cited	144
Chapter 4.....	148
Dietary supplementation with ovine serum immunoglobulins results in	

enrichment of <i>Lactobacillus johnsonii</i> in the growing rat.....	148
4.1. Abstract.....	149
4.2. Introduction	150
4.3. Materials and Methods	150
4.3.1. Preparation and quantitation of ovine serum Ig.....	150
4.3.2. Animal study.....	151
4.3.3. Chemical analysis.....	151
4.3.4. Post-mortem procedure	151
4.3.5. Isolation of bacterial DNA from digesta and PCR-DGGE ..	152
4.3.5.1. DGGE profile analysis	152
4.3.5.2. Sequencing of DGGE gel bands	153
4.3.5.3. <i>L. johnsonii</i> preparation and DNA extraction	153
4.3.5.4. Quantitative Real-Time PCR.....	155
4.3.6. Statistical analysis	155
4.4. Results	155
4.4.1. Bacterial communities in ileal digesta	155
4.4.2. Bacterial communities in colonic digesta	156
4.5. Discussion.....	157
4.6. Literature cited	161
Chapter 5.....	165
Dietary supplementation with ovine serum immunoglobulins is associated with increased gut mucin secretion in the growing rat	165
5.1. Abstract.....	166
5.2. Introduction	167
5.3. Materials and methods	168
5.3.1. Preparation and quantitation of ovine serum Ig.....	168
5.3.2. Animal study.....	169
5.3.3. Chemical analysis.....	169
5.3.4. Post-mortem procedure	169

5.3.5. Intact and cavitated goblet cell count	170
5.3.6. RNA extraction and quantitative real-time polymerase chain reaction.....	170
5.3.7. Enzyme-linked lectin assay for mucin	172
5.3.8. Statistical analysis.....	173
5.4. Results.....	173
5.4.1. Food intake and growth rate.....	173
5.4.2. Intact and cavitated goblet cell count	174
5.4.3. Mucin gene expression	174
5.4.4. Quantification of mucin protein.....	174
5.5. Discussion	177
5.6. Literature cited	182
Chapter 6.....	187
Dietary supplementation with ovine serum immunoglobulin influences growth performance, organ weight, gut morphology and intestinal mucin production in growing rats challenged with <i>Salmonella enteritidis</i>	187
6.1. Abstract	188
6.2. Introduction.....	189
6.3. Materials and methods.....	190
6.3.1. Preparation and quantitation of ovine serum Ig	190
6.3.2. Experimental diets	190
6.3.3. Animal study	192
6.3.4. Chemical analysis	193
6.3.5. Gut morphology	193
6.3.6. Intact goblet cell count	194
6.3.7. Enzyme-linked lectin assay for mucin	194
6.3.8. Statistical analysis.....	194
6.4. Results.....	195
6.4.1. Growth performance	195
6.4.2. Organ weights.....	195

6.4.3. Gut morphology	198
6.4.4. Goblet cell count.....	198
6.4.5. Quantification of luminal mucin protein	204
6.5. Discussion.....	205
6.6. Literature cited	209
Chapter 7	212
Immunomodulatory effects of ovine serum immunoglobulin in growing rats challenged with <i>Salmonella enteritidis</i>	212
7.1. Abstract.....	213
7.2. Introduction	214
7.3. Materials and methods	215
7.3.1. Preparation and quantitation of ovine serum Ig.....	215
7.3.2. Experimental diets	215
7.3.3. Animal study.....	216
7.3.4. Chemical analysis.....	216
7.3.5. Post-mortem procedure	216
7.3.6. Assessment of phagocytosis	216
7.3.7. Lymphocyte proliferation assay	217
7.3.8. Analysis of cytokines	217
7.3.9. Quantitative analysis of IgA, IgG and IgE	218
7.3.10. Quantitative analysis of salmonella specific IgA and IgG	218
7.3.11. Isolation of bacterial DNA from digesta and PCR-DGGE	219
7.3.12. Statistical analysis	219
7.4. Results	219
7.4.1. Hematology	220
7.4.2. PBL phagocytosis.....	220
7.4.3. Lymphocyte proliferation.....	221
7.4.4. Cytokine and Ig analysis in PP and spleen cell culture supernatants (ConA stimulated minus ConA unstimulated).....	222
7.4.5. Cytokine and Ig analysis in plasma.....	223

7.4.6. Ig analysis in intestinal digesta	223
7.4.7. Anti-S. enteritidis (ASE) Ig analysis in intestinal digesta	224
7.4.8. Bacterial communities in ileal and colonic digesta	225
7.5. Discussion	226
7.6. Literature cited	232
Chapter 8.....	237
Intact ovine serum immunoglobulin in the luminal digesta of the growing rat.....	237
8.1. Abstract	238
8.2. Introduction	239
8.3. Materials and methods.....	240
8.3.1. Animal trial	240
8.3.2. Post-mortem procedure	240
8.3.3. Quantitative analysis of intact ovine IgG.....	240
8.3.4. Qualitative analysis of intact and digested sheep IgG	241
8.4. Results.....	242
8.4.1. Food intake and growth rate.....	242
8.4.2. Quantitative analysis of intact ovine IgG.....	242
8.4.3. Qualitative detection of intact and digested ovine IgG	242
8.5. Discussion	243
8.6. Literature cited	246
Chapter 9.....	249
Overall Discussion and Conclusions.....	249
9.1. Discussion and conclusions	250
9.2. Future directions	262
9.3. Literature cited	263
Appendix.....	270
Appendix A - Nucleotide sequence data of excised DGGE bands (Chapter-4).....	271

A 1	Sample ID – IL-1 (AB544006).....	271
A 2	Sample ID – IL-2 (AB544007).....	271
A 3	Sample ID – CBD1 (AB544008)	271
A 4	Sample ID – CFD2 (AB544009).....	272
A 5	Sample ID – CFD3 (AB544010).....	272
A 6	Sample ID – CFD4 (AB544011).....	272
A 7	Sample ID – CFD5 (AB544012).....	273
Appendix B - Standard curve for <i>L. Johnsonii</i> ATCC 33200		274
Appendix C - Scatter plot of the 15 individual qPCR data (A), ileal digesta and (B), colonic digesta		275
Appendix D - Full length of each amplicon of mucin genes and the internal probes		276
D 1	Muc2.....	276
D 2	Muc3.....	276
D 3	Muc4.....	276
D 4	Muc 5AC.....	277
D 5	Beta-actin	277
Appendix E - Nucleotide sequence data of excised DGGE bands (<i>Chapter-7</i>)		278
E 1	Sample ID – ILFS1 (AB576354).....	278
E 2	Sample ID – ILFS2 (AB576355).....	278
E 3	Sample ID – ILFS3 (AB576356).....	278
E 4	Sample ID – ILFS4 (AB576357).....	279
E 5	Sample ID – ILFS5 (AB576358).....	279
E 6	Sample ID – CFS1 (AB576359)	279
E 7	Sample ID – CFS2 (AB576360)	279
E 8	Sample ID – CFS3 (AB576361)	280
E 9	Sample ID – CFS4 (AB576362)	280
E 10	Sample ID – CFS5 (AB576363)	281

LIST OF TABLES

Table 1.1 Reports of Ig recovery after oral administration.	58
Table 2.1 Ingredient compositions and determined nutrient and energy contents of the control and test diets.	112
Table 2.2 Protein and Ig concentrations of the Ig fractions and estimated Ig concentration in the test diets.	113
Table 2.3 Growth performance of rats fed diets containing ovine Ig for 21 d.	114
Table 2.4 Relative organ weights and empty body weight of rats fed diets containing ovine Ig for 21 d.	115
Table 2.5 Intestinal morphology of rats fed diets containing ovine Ig for 21 d ¹	117
Table 2.6 Relative organ weights of rats fed diets containing ovine Ig and SDPP for 21 d.	118
Table 2.7 Intestinal morphology of rats fed diets containing ovine Ig and SDPP for 21 d.	119
Table 3.1 Ingredient composition and determined nutrient and energy content of the control and test diets.	132
Table 3.2 Spleen lymphocyte proliferative responses (stimulation index) to ConA, LPS and PHA for rats fed a diet containing ovine Ig for 21 days.	137
Table 3.3 IFN γ and IL-4 production (pg/ml) by spleen lymphocyte cells from rats fed a diet containing ovine Ig for 21 days ¹ with and without ConA stimulation.	138
Table 3.4 IgA and IgG concentrations of intestinal digesta and plasma for rats fed a diet containing ovine Ig for 21 days.	139
Table 4.1 Identified bacterial species from DNA sequencing of the PCR-DGGE bands shown in Figure 4.1.	156
Table 5.1 Primers and probes for quantitative Real Time PCR.	172
Table 5.2 Goblet cell counts in the small intestine and colon for rats fed a diet	

containing ovine Ig for 21 d.....	175
Table 5.3 Cavitated goblet cell counts in the small intestine and colon for rats fed a diet containing ovine Ig for 21 d.	176
Table 5.4 Mucin protein in stomach chyme and ileal and colonic luminal digesta for rats fed a diet containing ovine Ig for 21 d.....	176
Table 6.1 Ingredient composition and determined nutrient and energy content of the control and test diets.....	191
Table 6.2 Growth performance of rats before and after challenging with <i>S. enteritidis</i>	196
Table 6.3 Mean (<i>n</i> =10) intestinal morphology characteristics in the growing rat after challenging with <i>S. enteritidis</i>	199
Table 6.4 Mean (<i>n</i> =10) goblet cell counts in the intestine of growing rats after challenging with <i>S. enteritidis</i>	204
Table 6.5 Mean (<i>n</i> =10) concentration of mucin protein in the ileal and colonic luminal digesta for the growing rat following challenging with <i>S. enteritidis</i>	205
Table 7.1 Mean haematological parameters (<i>n</i> =15) in the growing rat after challenging with <i>S. enteritidis</i>	220
Table 7.2 Phagocytic activity of peripheral blood leucocytes and proliferative responses of Peyer's patch cells and spleen cells to ConA and LPS in the unchallenged rats fed the BD diet and rats challenged with <i>S. enteritidis</i> and fed either the BD, FDOI or IOI diets.	221
Table 7.3 IFN γ , IL-4, IgA and IgG production by ConA stimulated Peyer's patch cells and spleen cells in unchallenged rats fed the BD diet and rats challenged with <i>S. enteritidis</i> * and fed either the BD, FDOI or IOI diets.....	222
Table 7.4 IFN γ , IL-4, IL-10, TNF α , IgA, IgG, and IgE concentrations in plasma for the unchallenged rats fed the BD diet and rats challenged with <i>S. enteritidis</i> * and fed either the BD, FDOI or IOI diets.....	223
Table 7.5 Ig concentrations in intestinal digesta for unchallenged rats fed the BD diet and rats challenged with <i>S. enteritidis</i> and fed either the BD, FDOI or IOI diets.	224
Table 7.6 Concentrations of specific anti- <i>S. enteritidis</i> IgA and IgG in	

intestinal digesta and plasma for unchallenged rats fed the BD diet and rats challenged with *S. enteritidis* and fed either the BD, FDOI or IOI diets.225

Table 7.7 Identified bacterial species from DNA sequencing of the PCR-DGGE bands for the challenged FDOI-fed rats (refer Figure 7.1).226

Table 8.1 Amounts of intact ovine IgG in stomach chyme and intestinal digesta from growing rats fed with a diet containing ovine Ig.243

LIST OF FIGURES

Figure 1.1 Schematic diagram of intestinal epithelium.	5
Figure 1.2 The intestinal epithelial cell lining.	9
Figure 1.3 Gut barrier.	13
Figure 1.4 General structure of immunoglobulin.	46
Figure 1.5 Immunoglobulin digestion by enzymes.	53
Figure 3.1 Phagocytic activity of peripheral blood leukocytes in rats fed a diet containing ovine Ig for 21 days.	136
Figure 4.1 Lanes showing DGGE bands from pooled DNA samples ($n=15$) of ileal (A) and colonic (B) digesta used for sequencing for the identification of bacteria in rats fed a basal diet or a diet containing ovine Ig for 21 days.	157
Figure 4.2 Quantification of <i>L. johnsonii</i> strains using quantitative real-time PCR in ileal and colonic digesta of each diet group.	158
Figure 5.1 Transverse sections of rat ileum.	178
Figure 5.2 Expression of mRNA levels of mucin genes (Muc5Ac, Muc2, Muc3 and Muc4) in the stomach, ileum and colon of rats fed a diet containing ovine Ig for 21 d.	179
Figure 6.1 Mean ($n=15$) relative organ weights (% empty body weight) for the growing rat fed diet BD and the growing rat with <i>S. enteritidis</i> and fed either diet BD, FDOI or IOI-supplemented diets.	197
Figure 6.2 Transverse sections of rat duodenum. Section was stained with haemotoxylin, eosin and alcian blue.	200
Figure 6.3 Transverse sections of rat jejunum. Section was stained with haemotoxylin, eosin and alcian blue.	201
Figure 6.4 Transverse sections of rat ileum. Section was stained with haemotoxylin, eosin and alcian blue.	202
Figure 6.5 Transverse sections of rat colon. Section was stained with haemotoxylin, eosin and alcian blue.	203
Figure 7.1 Lanes showing DGGE bands from pooled DNA samples ($n=15$) of ileal (A) and colonic (B) digesta used for sequencing for the	

identification of bacteria in unchallenged rats fed the BD diet and rats challenged with *S. enteritidis* and fed either the BD, FDOI or IOI diets. Bands were selected for sequencing only when they were showing an obvious difference between the diets. 227

Figure 8.1 Detection of intact ovine IgG in chyme and intestinal digesta for rats fed a diet containing ovine IgG for 21 d. 244

LIST OF ABBREVIATIONS

AA – Amino acid

ADCC – Antibody dependent cell-mediated cytotoxicity

ADFI – Average daily feed intake

ADG – Average daily gain

AMI – Antibody-mediated immunity

AP – Animal plasma

BD – Basal diet

BSA – Bovine serum albumin

C3b – complement component 3

cAMP – Cyclic adenosine monophosphate

cGMP – Cyclic guanosine monophosphate

CD – Crohn's disease

CD4 – cluster of differentiation 4

CD8 – cluster of differentiation 8

cDNA – complementary DNA

CDR – complementarity determining regions

CFU – Colony forming unit

C_H – Constant heavy chain

C_L – Constant light chain

CLR – C-type lectin receptor

Con A – Concanvallin A

CPM – Counts per minute

CT – cholera toxin

CXC R1 – chemokine receptor 1

d – day

DC – Dendritic cells

DGGE – Denaturing Gradient Gel Electrophoresis

DNA – Deoxyribonucleic acid

dsRNA – double-stranded RNA viruses

EAggEC – enteroaggregative *E. coli*

EDTA –Ethylenediaminetetraacetic acid

EHEC– enterohemorrhagic *E. coli*

ELISA – Enzyme Linked Immunosorbent Assay

ELLA – Enzyme-Linked Lectin Assay

EPEC – enteropathogenic *E. coli*

ETEC – enterotoxigenic *E. coli*

Fab – “Fragment, antigen-binding”

FACS – Fluorescence-activated cell sorter

FAE – Follicle-Associated Epithelium

Fc – “Fragment, crystallisable”

FcR – Fc receptor

FCγbp – Fc-gamma binding protein

FDOI – Freeze dried ovine immunoglobulin

FOS – fructo-oligosaccharides

GALT – Gut associated lymphoid tissue

G:F – Gain:feed ratio

GIT – Gastrointestinal tract

GOS – galacto-oligosaccharides

H₂O₂ – hydrogen peroxide

HD – human defensins

HIP / PAP – hepatocarcinoma-intestine-pancreas / pancreatic-associated protein

HRP – Horseradish peroxidase

HSP – Heat shock protein

HVR – Hyper variable region

IBD – Inflammatory bowel disease

IEC – intestinal epithelial cells

IFN γ – Interferon gamma

Ig – Immunoglobulin/ Immunoglobulins

IgA – Immunoglobulin A

IgD – Immunoglobulin D

IgE – Immunoglobulin E

IgG – Immunoglobulin G

IgM – Immunoglobulin M

IgY – Egg yolk antibodies

IL-1 – Interleukin -1

IL-1R – Interleukin -1 receptor

IMO – isomalto-oligosaccharide

iNOS – inducible nitric oxide synthase

IOI – Inactivated ovine immunoglobulin

IPS-1 – Interferon promoter stimulator-1

JAM – junctional adhesion protein

kDa – kiloDalton

LEAPs – liver-expressed antimicrobial peptides

LPS – lipopolysaccharide

LT – Heat Labile Toxin

LTB₄ – leucotrien B 4

M-SAA3 – mammary-associated serum amyloid A isoform 3

MALT – mucosal-associated lymphoid tissue

M-cell – Microfold cells

MDA5 – Melanoma differentiation associated gene 5

MHC – Major histocompatibility complex

mRNA – messenger ribonucleic acid

MUC – Mucin genes (human)

Muc – Mucin genes (rat)

NA – Natural antibodies

NF- κ B – Nuclear factor kappa B

NLR – Nucleotide-binding domain leucine-rich repeat

NK cells – Natural killer cells

NO – Nitric oxide

NOD – Nucleotide-binding domain

NOS – nitric oxide synthase

O₂⁻ – Superoxide anion

OIC – Ovine serum immunoglobulin concentrate

OPD – o-phenylenediamine dihydrochloride

PAMPs – Pathogen-associated molecular patterns

PBL – Peripheral blood leukocytes

PBS-T – Phosphate buffer saline–tween 20

PCR – Polymerase chain reaction

PHA – Phytphaemagglutinin

pIgR – Polymeric immunoglobulin receptor

PLA₂ – Phospholipase A₂

PP – Peyer's patch

PRR – pattern recognition receptors

qRT-PCR – Quantitative Real Time –Polymerase Chain Reaction

RELM β – resistin-like molecule beta

RLR – Rretinoic-acid-inducible protein 1–like receptors

RNA – Ribonucleic acid

s – Seconds (time)

SAP130 – Sin3-associated polypeptide p130

SDAP / SDBP – Spray dried animal plasma / Spray dried bovine plasma

SDOI – Spray dried ovine immunoglobulin

SDP – Spray dried plasma

SDPP – Spray dried porcine plasma

SDS-PAGE – sodium dodecyl sulphate polyacrylamide gel electrophoresis

SEB – *Staphylococcus aureus* enterotoxin B

SED – subepithelial dome

SEM – Standard error of mean

SIgA – Secretory immunoglobulin A

SIgM – Secretory immunoglobulin G

ST – Stable enterotoxins

TER – transepithelial electric resistance

TFP – trefoil factor peptides

TGF β – Transforming growth factor

T_H1 – T helper cells 1

T_H2 – T helper cells 2

T_H17 – T helper cells 17

TLR – Toll like receptor

TNF α – Tumour necrosis factor alpha

UC – Ulcerative colitis

VR – Variable region

VSR – Villous surface area

WGA – Wheat germ agglutinin

Wk – Week

ZO – *zonula occludens*

Zot – *Zonula occludens* toxin

LIST OF PUBLICATIONS

Peer-reviewed articles published or in progress

1. **P Balan**, KS Han, K Rutherford-Markwick, H Singh and PJ Moughan. (2011). Immunomodulatory effects of ovine serum immunoglobulin in growing rats gavaged with *Salmonella enteritidis* J Nutr. 141, 950-956.
2. **P Balan**, Kyoung-Sik Han, H Singh and PJ Moughan. (2011). Dietary supplementation with ovine serum immunoglobulin is associated with increased gut mucin secretion in the growing rat. (Animal). DOI:10.1017/S1751731111001108.
3. **P Balan**, KS Han, SM Rutherford, H Singh and PJ Moughan. (2011). Dietary supplementation with ovine serum immunoglobulin attenuates acute effects on growth, organ weights, gut morphology and intestinal mucin production in the growing rat challenged with *Salmonella enteritidis*. Animal. 5, 1570-1578
4. KS Han, **P Balan**, F Gasa and M Boland. (2011). Green kiwifruit modulates the colonic microbiota in growing pigs. Lett Appl Microbiol. 52, 379-385.
5. **P Balan**, KS Han, K Rutherford-Markwick, H Singh and PJ Moughan. (2010) Immunomodulatory effects of ovine serum immunoglobulin in the growing rat. Animal. 4, 1702-1708.
6. **P Balan**, KS Han, SM Rutherford, H Singh and PJ Moughan. (2009) Orally administered ovine serum immunoglobulins influence growth performance, organ weights, and gut morphology in growing rats. J Nutr. 139, 244-9.

Confidential reports

7. SJ Henare, SM Rutherford, M Zou, KS Han, **P Balan**, N Strobinger, S Saigeman, T Olson, C Sawatdeenaruenat, A Purba, MJ Boland and PJ Moughan. (2011). Gastrointestinal interactions of kiwifruit: Effects on mucin production in piglets
8. SM Rutherford, TK Chung, DV Thomas, ML Zou, KS Han, **P Balan**, E Maier and PJ Moughan (2011). Effect of microbial phytase on broiler performance, AME, toe ash, bone mineral density, ileal and colonic microbial population, gut mucin production and the digestibility of phytase P, minerals and amino acids.

Manuscripts ready for submission

9. **P Balan**, KS Han, B Lawley, H Singh and PJ Moughan. Orally administered ovine serum immunoglobulins modulate the levels of *Lactobacillus* and *Enterobacteria* in the growing rat.
10. **P Balan**, KS Han and PJ Moughan. Recovery of intact immunoglobulin in the digesta of the growing rat following ingestion of an ovine serum immunoglobulin.

Manuscripts in preparation

11. **P Balan** and PJ Moughan. Immunoglobulins – Review
12. **P Balan** and PJ Moughan. Ovine serum immunoglobulin supplements prevent the release of mucosal proinflammatory mediators in the growing rat challenged with *Salmonella enteritidis*.
13. **P Balan** and PJ Moughan. Stimulatory effect of ovine serum Ig on multiplication of lactic acid bacteria under *in vitro* condition.
14. **P Balan**, SM Rutherfurd and PJ Moughan. Effects of ovine serum immunoglobulin on dental health and immunomodulation in the cat.
15. **P Balan**, G Mal, S Das and PJ Moughan. Synergistic antimicrobial activity of curcumin, manuka honey and whey protein isolate.
16. KS Han, **P Balan**, A Purba and PJ Moughan. Effect of Korean ginsengs on gut microbiota and mucin secretion in growing rats.
17. KS Han, **P Balan**, A Purba and PJ Moughan. Effect of Korean traditional foods-derived polysaccharides on lymphocyte proliferation of Peyer's patch, cytokine and immunoglobulin production in the ileum of growing rats.
18. SM Rutherfurd, TK Chung, **P Balan**, KS Han, E Maier and PJ Moughan. Effect of three microbial phytases on mucin output and gut microbiota in broilers fed low-phosphorus corn-soybean diets.
19. SJ Henare, SM Rutherfurd, M Zou, **P Balan**, KS Han, N Strobinger, MJ Boland and PJ Moughan. Gastrointestinal interactions of kiwifruit: Effects on mucin production in piglets.
20. SM Rutherfurd, SJ Henare, RK Richardson, ML Zou, **P Balan**, C Sawatdeenaruenat and PJ Moughan. The effect of dietary protein content on endogenous ileal tryptophan flow in the growing rat.

Patent application

21. **P Balan**, Shane M Rutherford, H Singh and PJ Moughan. Ovine serum immunoglobulins positively modulate dental health and immunity in the cat.

Abstract, conference and other presentations

22. **P Balan**. Ovine serum Immunoglobulins – *In vitro* and *In vivo* studies. Oral presentation at the Research day, Riddet Institute, Massey University, Palmerston North, New Zealand, August 2011.
23. **P Balan** and PJ Moughan. Potential application of ovine serum immunoglobulins during total parenteral nutrition. Poster presentation at the International Symposium: Dietary Protein for Human Health Auckland 1142, New Zealand, March 2011.
24. **P Balan**, KS Han, H Singh, and PJ Moughan. Orally administered ovine serum immunoglobulins modulates the immunity and gut function in the growing rat. Oral presentation at the CORE meeting, Palmerston North, New Zealand, June 2010.
25. **P Balan**, KS Han, H Singh and PJ Moughan. Gut microbial modulation and immunomodulation by ovine serum immunoglobulins in the growing rat. Oral presentation at the USA/IRELAND functional food conference, Cork, Ireland, March 2010.
26. **P Balan**, KS Han, H Singh and PJ Moughan. Gut microbial modulation and immunomodulation by ovine serum immunoglobulins in the growing rat. Oral and poster presentation at the PhD student Colloquium, Palmerston North, New Zealand, October 2009.
27. **P Balan**, KS Han, H Singh and PJ Moughan. Immunomodulation of ovine serum immunoglobulins in the growing rat. Poster presentation at the Functional food conference, Riddet Institute, Palmerston North, New Zealand, February 2009.
28. KS Han, R Sengupta, **P Balan**, A Deglaire, H Singh and PJ Moughan. Effect of bioactive protein on mucin gene expression in rat small intestine. Poster presentation at the 17th Queenstown Molecular Biology Meeting. Queenstown, New Zealand. 2007.