UNIVERSITI PUTRA MALAYSIA

IN VITRO AND IN VIVO EFFECTS OF PALM KERNEL CAKE OLIGOSACCHARIDES ON Salmonella enterica SEROVAR ENTERITIDIS INFECTION

FOO RUI QING

IPTSM 2021 10

IN VITRO AND *IN VIVO* EFFECTS OF PALM KERNEL CAKE OLIGOSACCHARIDES ON *Salmonella enterica* SEROVAR ENTERITIDIS INFECTION

By

FOO RUI QING

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

March 2021

COPYRIGHT

DEDICATIONS

This thesis is dedicated to my parents whose love, encouragement, belief, patience and unending support made this endeavour possible.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

IN VITRO AND _IN VIVO_ EFFECTS OF PALM KERNEL CAKE OLIGOSACCHARIDES ON _Salmonella enterica_ SEROVAR ENTERITIDIS INFECTION

By

FOO RUI QING

March 2021

Chair: Liang Juan Boo, PhD
Institute: Tropical Agriculture and Food Security

Non-digestible oligosaccharides (NDOs) have been reported to possess prebiotic and immunomodulatory properties. With the European Union (EU) wide ban on the use of antibiotic growth promoters (AGP) in the livestock industry, the search for AGP alternatives to maintain livestock productivity is highly sought after. Previous studies have shown that NDOs from palm kernel cake (PKC), a by-product of the palm oil industry were able to confer a protective effect against *Salmonella* colonization in young chickens. Being young vertebrae with an immature immune system, they would have to rely on the strength of their innate immune responses to prevent *Salmonella* infection. Therefore the objective of this study was to investigate the ability of NDOs from PKC to affect the innate immune response in a *Salmonella enterica* serovar (ser.) Enteritidis (*S.* Enteritidis) infection model utilizing *in vitro* and *in vivo* approaches. The *in vitro* approach consisted of an anti-adherence assay using Caco-2 cells, a *Salmonella* killing assay using U-937 macrophages and a lactate dehydrogenase (LDH) assay to monitor cellular damage. The *in vivo* approached utilized zebrafish larvae to observe the effects of NDOs from PKC on lipopolysaccharide (LPS) induced nitric oxide (NO) levels, *S.* Enteritidis colonization patterns and its effect on zebrafish's gene expression. The results of the *in vitro* study revealed that the NDOs fraction from PKC with a lower degree of polymerization (DP), termed 'Small' (DP ≤ 6), was better than the NDOs fraction from PKC with a larger DP, termed 'Big' (DP > 6), at significantly ($p \leq 0.05$) reducing S. Enteritidis adherence to Caco-2 epithelial cells. Both Small and Big fractions were comparable to one another at increasing the rate of *Salmonella* killing in U-937 macrophages. In terms of reducing cellular damage, both the Small and Big were capable of significantly reducing cellular damage in Caco-2 cells although the Small fraction showed a stronger correlation between decreasing *S.* Enteritidis numbers and cellular damage in U-937 macrophages than the Big fraction. When compared to commercial NDOs fructooligosaccharide (FOS) and mannanoligosaccharide (MOS), both the Small and Big fraction were found to be comparable, if not better

than MOS and FOS at reducing *S.* Enteritidis adherence, increasing *S.* Enteritidis elimination and reducing cellular damage *in vitro*. As the Small fraction was better than Big, it was chosen for subsequent *in vivo* studies and renamed 'OligoPKC'. The results of the *in vivo* study showed that OligoPKC was comparable to the commercial NDOs at reducing LPS induced NO levels in zebrafishes but showed a significant increase in *S.* Enteritidis colonization within the gastrointestinal tract at 24 hours postinfection when compared to the infected control. This might stem from a weaker upregulation of *myeloperoxidase (MPX)* expression when compared to other infected groups. In conclusion, NDOs from PKC were able to affect the innate immune response in a *S.* Enteritidis infection model *in vitro* and *in vivo* but care should be taken when selecting the appropriate animal infection model and interpreting its results.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN *IN VITRO* DAN *IN VIVO* HAMPAS ISIRUNG KELAPA SAWIT TERHADAP JANGKITAN *Salmonela enterica* SEROVAR ENTERITIDIS

Oleh

FOO RUI QING

Mac 2021

Pengerusi: Liang Juan Boo, PhD
Institut: Pertanian Tropika dan Sekuriti Makanan

Oligosakarida yang tidak dicerna (NDOs) dilaporkan mempunyai sifat prebiotik dan imunomodulator. Dengan larangan meluas Kesatuan Eropah (EU) terhadap penggunaan penggalak pertumbuhan antibiotik (AGP) dalam industri ternakan, pencarian alternatif AGP untuk mengekalkan produktiviti ternakan perlu dicari. Hampas isirung kelapa sawit (PKC) ialah produk sampingan industri kelapa sawit. Kajian terdahulu menunjukkan bahawa NDOs daripada PKC boleh memberikan kesan perlindungan kepada anak ayam daripada jangkitan *Salmonela*. Sebagai vertebra muda dengan sistem imun yang belum matang, anak-anak ayam ini sememangya bergantung kepada kekuatan tindak balas imun semula jadi mereka untuk mencegah jangkitan *Salmonela*. Oleh itu objektif kajian ini adalah untuk mengkaji kemampuan NDOs daripada PKC untuk mempengaruhi tindak balas imun semula jadi dalam model jangkitan *Salmonela enterica* serovar (ser.) Enteritidis (*S*. Enteritidis) melalui kaedah *in vitro* dan *in vivo*. Kajian *in vitro* terdiri daripada ujian untuk mengurangkan pelekatan *S.* Enteritidis ke atas sel epitelium usus Caco-2, ujian pembasmian patogen *Salmonela* menggunakan sel makrofaj U-937 dan ujian laktat dehidrogenase (LDH) untuk memantau tahap kerosakan sel. Kajian *in vivo* larva zebrafish digunakan untuk memerhatikan kesan NDO daripada PKC pada tahap nitrik oksida (NO) yang dicetuskan oleh lipopolisakarida (LPS), corak pengkolonian *S*. Enteritidis dan kesannya terhadap ekspresi gen zebrafish. Hasil kajian *in vitro* menunjukkan bahawa NDOs daripada PKC dengan tahap pempolimeran (DP) yang lebih rendah, yang dipanggil 'Kecil' (DP ≤ 6), lebih baik daripada NDOs daripada PKC dengan DP yang lebih besar, dipanggil 'Besar '(DP> 6), dari segi mengurangkan pelekatan *S.* Enteritidis pada sel epitelium Caco-2 dengan signifikan ($p \leq 0.05$). Kedua-dua pecahan Kecil dan Besar dapat dibandingkan antara satu sama lain dalam meningkatkan kadar pembasmian *Salmonela* dalam sel makrofaj U-937. Dari segi mengurangkan kerosakan sel, kedua-dua pecahan Kecil dan Besar mampu mengurangkan kerosakan sel secara signifikan pada sel Caco-2 walaupun pecahan Kecil menunjukkan korelasi yang lebih kuat antara penurunan bilangan *S*. Enteritidis dan kerosakan sel pada

makrofag U-937 daripada pecahan Besar . Jika dibandingkan dengan NDOs komersial fruktooligosakarida (FOS) dan mannanoligosakarida (MOS), kedua-dua pecahan Kecil dan Besar didapati setanding, jika tidak lebih baik daripada MOS dan FOS untuk mengurangkan pelekatan *S.* Enteritidis, meningkatkan kadar pembasmian *S*. Enteritidis dan mengurangkan kerosakan sel secara *in vitro*. Oleh sebab pecahan Kecil lebih baik daripada Besar, ia dipilih untuk kajian *in vivo* selanjutnya dan dinamakan semula sebagai "OligoPKC". Hasil kajian *in vivo* menunjukkan bahawa OligoPKC setanding dengan NDO komersial dalam mengurangkan kadar NO yang disebabkan oleh LPS pada zebrafish tetapi menunjukkan peningkatan yang signifikan dalam kolonisasi S. Enteritidis dalam saluran gastrousus pada 24 jam selepas jangkitan apabila dibandingkan dengan jangkitan kumpulan kawalan. Ini mungkin berpunca daripada regulasi ekspresi mieloperoksidase (MPX) yang lebih lemah jika dibandingkan dengan kumpulan lain yang dijangkiti. Sebagai kesimpulan, NDOs daripada PKC dapat mempengaruhi tindak balas imun semula jadi dalam model jangkitan *S.* Enteritidis *in vitro* dan *in vivo* tetapi perlu berhati-hati ketika memilih model jangkitan haiwan yang sesuai dan menafsirkan keputusanya.

ACKNOWLEDGEMENTS

First and foremost I would like to thank the members of my supervisory committee; Dr. Liang Juan Boo, Prof. Zulkifli Bin Idrus, Assoc. Prof. Dr. Syahida Binti Ahmad, and Dr. Lai Kok Song; for the opportunity to grow and learn from them. Their vast experience, patience, and insights were crucial in broadening my mind, shaping my approach to problem solving, and decision making. I feel honoured to have been under their tutelage.

Secondly, I would like to thank the members of the Medical Biotechnology Research Group (MBRG). The camaraderie, suggestions and feedbacks made research interesting and exciting.

Thirdly I would like to thank En. Rusdam and En. Kamaruddin from the Faculty of Veterinary Medicine for taking the time to teach me cell culture techniques. In addition, I would like to thank En. Faizol and Pn. Ummi from the Laboratory of Animal Production for accommodating my oftentimes erratic working schedule. Special thanks also goes to the staff of Agro-Biotechnology Institute Malaysia (ABI) for their technical assistance.

Last but not least, I would like to thank my friends and family for their support, encouragement and time without which this journey would be insurmountable. To my laboratory friends, your dedication and tenacity to research has been nothing short of inspirational.

This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Liang Juan Boo, PhD
Research Fellow
Institute of Tropical Agriculture and Food Security
Universiti Putra Malaysia
(Chairman)

Zulkifli Bin Idrus, PhD
Professor
Institute of Tropical Agriculture and Food Security
Universiti Putra Malaysia
(Member)

Syahida Binti Ahmad, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Lai Kok Song, PhD
Assistant Professor
Health Sciences Division
Abu Dhabi Women's College
Higher Colleges of Technology, UAE
(Member)

_____
ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 12 August 2021

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotation, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of the thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: Foo Rui Qing, GS42192

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of Chairman
of Supervisory
Committee: _____Dr. Liang Juan Boo_____

Signature _____
Name of Member
of Supervisory
Committee: _____Prof. Zulkifli Bin Idrus_____

Signature: _____
Name of Member
of Supervisory
Committee: _____Assoc. Prof. Dr. Syahida Binti Ahmad_____

Signature: _____
Name of Member
of Supervisory
Committee: _____Dr. Lai Kok Song_____

TABLE OF CONTENTS

		Page
ABSTRACT		i
ABSTRAK		iii
ACKNOWLEDGEMENTS		v
APPROVAL		vi
DECLARATION		viii
LIST OF TABLES		xiii
LIST OF FIGURES		xv
LIST OF APPENDICES		xvii
LIST OF ABBREVIATIONS		xviii
CHAPTER		
1	**INTRODUCTION**	1
	1.1 Problem statement and justification	4
	1.2 Hypothesis	4
	1.3 Objectives	4
2	**LITERATURE REVIEW**	6
	2.1 Oligosaccharides as prebiotics	6
	2.2 Structure and properties of NDOs	7
	2.3 Mechanism of prebiotic oligosaccharide action	9
	2.4 Sources and production of prebiotic oligosaccharides	10
	2.5 Commercial production of prebiotics	12
	2.6 Prebiotics in the livestock industry	13
	2.6.1 Poultry	14
	2.6.2 Ruminant, swine and fishing industry	16
	2.7 Waste to wealth concept	17
	2.8 PKC as a potential source of non-digestible oligosaccharides	18
	2.9 Immune recognition of carbohydrates	18
	2.10 The gut immune system	19
	2.11 Role of cytokines	20
	2.12 *Salmonella*	21
	2.13 Cell culture as an *in vitro* model for *Salmonella* infection	22
	2.14 The use of animal models for *Salmonella* infection	23
	2.15 Zebrafish	24
	2.16 Summary	26
3	**OLIGOSACCHARIDES FROM PALM KERNEL CAKE ENHANCES ADHERENCE INHIBITION AND**	27

INTRACELLULAR CLEARANCE OF *SALMONELLA* ENTERICA SEROVAR ENTERITIDIS *IN VITRO*
3.1 Introduction 27
3.2 Materials and methods 29
3.2.1 Sample preparation 29
3.2.2 Size exclusion chromatography 30
3.2.3 Pooling fractions based on HPLC profiles 30
3.2.4 Molecular weight determination 31
3.2.5 Cells and bacterial culture conditions 32
3.2.6 Adherence inhibition study using Caco-2 cells 32
3.2.7 LDH analysis of Caco-2 cells 33
3.2.8 Intracellular *Salmonella* clearance using U-937 macrophages 34
3.2.9 Statistical analysis 34
3.3 Results 35
3.3.1 HPLC profiles of PKC oligosaccharides 35
3.3.2 Molecular weights of oligosaccharides 36
3.3.3. *Salmonella* Enteritidis adherence inhibition to Caco-2 cells 36
3.3.4 Effects of oligosaccharides on the release of LDH in LPS-induced Caco-2 cells 37
3.3.5 Intracellular clearance of *Salmonella* Enteritidis in U-937 macrophages 38
3.3.6 LDH measurement and correlation between LDH levels and intracellular *Salmonella* Enteritidis clearance in U-937 when treated with non-digestible oligosaccharides 39
3.4 Discussion 41
3.5 Conclusion 44

4 PALM KERNEL CAKE OLIGOSACCHARIDES ACUTE TOXICITY AND EFFECTS ON NITRIC OXIDE LEVELS USING A ZEBRAFISH LARVAE MODEL 45
4.1 Introduction 45
4.2 Materials and methods 47
4.2.1 OligoPKC preparation 47
4.2.2 Zebrafish care and maintenance 47
4.2.3 Zebrafish embryo acute toxicity test 47
4.2.4 *In vivo* measurement of nitric oxide levels in zebrafish larvae 48
4.2.5 Statistical analysis 48
4.3 Results 49
4.3.1 Acute toxicity assay 49

4.3.1.1 Survival rate 49
4.3.1.2 Hatching rate 51
4.3.1.3 Malformation effects 53
4.3.2 Measurement of *in vivo* nitric oxide levels 55
4.4 Discussion 56
4.5 Conclusion 58

5 EFFECT OF OLIGOSACCHARIDES FROM PALM KERNEL CAKE ON *SALMONELLA ENTERICA* SEROVAR (SER.) ENTERITIDIS COLONIZATION AND GENE EXPRESSION IN ZEBRAFISH LARVAE 59
5. 1 Introduction 59
5.2 Materials and methods 62
5.2.1 OligoPKC preparation 62
5.2.2 Bacterial culture conditions 62
5.2.3 Zebrafish care and maintenance 63
5.2.4 *Salmonella* Enteritidis challenge 64
5.2.5 Live-cell imaging and quantifying of GFP labelled *Salmonella* Enteritidis in zebrafish larvae 65
5.2.6 RNA extraction and cDNA synthesis 65
5.2.7 Relative gene expression analysis 66
5.2.8 Statistical analysis 67
5.3 Results 67
5.3.1 Live-cell imaging of GFP labeled *Salmonella* Enteritidis in zebrafish larvae 67
5.3.2 Quantification of *Salmonella* Enteritidis in zebrafish larvae 68
5.3.3 Relative gene expression analysis 69
5.4 Discussion 71
5.5 Conclusion 73

6 GENERAL DISCUSSION 74
6.1 Summary 74
6.2 General conclusion 76
6.3 Limitations and recommendations for future research 77
6.4 Potential application of the technology 79

REFERENCES 81
APPENDICES 112
BIODATA OF STUDENT 132
LIST OF PUBLICATIONS 133

LIST OF TABLES

Table		Page
2.1	Sources of oligosaccharides	11
2.2	Commercial uses of various prebiotics	12
3.1	Molecular weight of monosaccharides and oligosaccharides found in Small, Big, MOS and FOS	36
3.2	Correlation between intracellular *Salmonella* Enteritidis in U-937 macrophages and LDH released.	41
5.1	Treatment groups for zebrafish larvae and their designated names	64
5.2	Primers and the annealing temperature used for quantitative real-time polymerase chain reaction	66
A2.1	Fold changes of fluorescent intensities in zebrafish larvae in response to different concentrations of LPS at 5 dpf.	114
A3.1	Fold changes of fluorescent intensities in zebrafish larvae in response to different treatment groups of MOS and FOS at 7 dpf.	115
A4.1	RNA quantification and purity check using Nanodrop ND-1000	118
A8.1	Shapiro-Wilk test for normality for survival rates of zebrafish larvae after 24 hours of exposure to OligoPKC.	124
A8.2	Shapiro-Wilk test for normality for hatching rates of zebrafish larvae after 48 hours of exposure to OligoPKC.	124
A8.3	Shapiro-Wilk test for normality for fold changes of fluorescence intensity of zebrafish larvae at 7 dpf treated with OligoPKC, MOS, FOS, a combination of different oligosaccharides and LPS.	124
A8.4	Shapiro-Wilk test for normality for fold changes of fluorescent intensities in zebrafish larvae in response to different treatment groups of MOS and FOS at 7 dpf.	125
B1.1	Absorbance value for the effects of oligosaccharide treatment on LDH release in LPS induced Caco-2 cells.	126

B2.1 Log CFU/mL values for the effect of oligosaccharide on the rate of intracellular *S*. Enteritidis clearance in U-937 macrophages. 127

B3.1 Absorbance value from 0 to 9 hours post infection for the effect of oligosaccharides on LDH released by U-937 macrophages infected with *S*. Enteritidis. 128

B3.2 Absorbance value from 14 to 18 hours post infection for the effect of oligosaccharides on LDH released by U-937 macrophages infected with S. Enteritidis. 129

B4.1 Fold changes of fluorescent intensities in zebrafish larvae in response to different treatment groups. 130

LIST OF FIGURES

Figure		Page
2.1	Chemical structure of amylose and cellulose	7
2.2	Chemical structure of FOS and GOS	8
2.3	Chemical structure of α-1, 3-mannobiose and β-1,4-mannobiose	9
2.4	Production quantity of poultry meat. (Source: FAOSTAT, 2015)	14
3.1	(a) High-Performance Liquid Chromatography (HPLC) profiles of oligosaccharide standards ranging from a DP of two to six.	35
3.2	Effect of oligosaccharide treatment on the percentage inhibition of *Salmonella* Enteritidis adherence to Caco-2 cells as compared to the control.	37
3.3	Effects of oligosaccharide treatment on lactate dehydrogenase (LDH) release in lipopolysaccharide (LPS)-induced Caco-2 cells.	38
3.4	Effect of (a) Small; (b) Big; (c) MOS; and (d) FOS on the rate of intracellular *S*. Enteritidis clearance in U-937 macrophages.	39
3.5	Effect of (a) Small; (b) Big; (c) MOS; and (d) FOS on LDH released by U-937 macrophages infected with *S*. Enteritidis.	40
4.1	Survival rates of zebrafish embryos treated with different concentrations of (a) OligoPKC; (b) MOS; and (c) FOS.	50
4.2	Hatching rates of zebrafish embryos treated with different concentrations of (a) OligoPKC; (b) of MOS and; (c) FOS.	52
4.3	Morphological characteristics of zebrafish larvae after 120 hours of exposure to OligoPKC.	54
4.4	Fluorescence intensity of zebrafish larvae at 7 dpf treated with OligoPKC, MOS, FOS, a combination of different oligosaccharides and LPS.	56
5.1	Effect of NDOs on *S*. Enteritidis colonization patterns in the gastrointestinal tract of zebrafish larvae at 7 and 8 dpf.	68

5.2	Quantification of S. Enteritidis in zebrafish larvae at 7 and 8 dpf with different NDOs treatment.	69
5.3	The effects of different NDOs on the gene expression of zebrafish larvae challenged with *S*. Entertitidis.	70
A1.1	Graph of survival rate (%) of zebrafish larvae after 120 hours of exposure versus base 10 logarithm (log_{10}) of OligoPKC.	112
A2.1	Effect of different levels of LPS on the fluorescent intensities (indicative of NO levels) in the heart region (encircled in white) of zebrafish larvae at 5 dpf with a 4x objective lens magnification.	113
A2.2	Preliminary study involving the measurement of fluorescence intensity of zebrafish larvae at 5 dpf which corresponds to different concentrations of LPS from Appendix Figure 2.1 and Appendix Table 2.1.	114
A3.1	Fluorescence intensity of zebrafish larvae at 7dpf treated with MOS, FOS, a combination of different oligosaccharides and LPS.	117
A5.1	Visual check on RNA integrity of Replicate 1 using 1% TAE agarose gel containing 1% (v/v) bleach (Clorox) and stained with FloroSafe DNA Stain (Axil Scientific Pte. Ltd., Singapore).	119
A5.2	Visual check on RNA integrity of Replicate 2 using 1% TAE agarose gel containing 1% (v/v) bleach (Clorox) and stained with FloroSafe DNA Stain (Axil Scientific Pte. Ltd., Singapore).	120
A5.3	Visual check on RNA integrity of Replicate 3 using 1% TAE agarose gel containing 1% (v/v) bleach (Clorox) and stained with FloroSafe DNA Stain (Axil Scientific Pte. Ltd., Singapore).	121
A6.1	Assessment of primer efficiency using standard curves.	122
A7.1	Melt curve analysis of RNA samples.	123

LIST OF APPENDICES

Appendix		Page
A1	Estimated LC50 Value of OligoPKC for Zebrafish Larvae Based on the Graphical Method	112
A2	Effect of Different Levels of LPS on Zebrafish Fluorescent Intensities	113
A3	Effect of MOS and FOS at 1000 µg/mL on Zebrafish Fluorescent Intensities	115
A4	RNA Quantification and Purity Check Using Nanodrop ND-1000	118
A5	Gel Electrophoresis of Zebrafish RNA Samples	119
A6	Primer Standard Curves and Efficiencies	122
A7	Melting Curve Analysis of Zebrafish Gene Expression	123
A8	Shapiro-Wilk Test for Normality	124
B1	Raw Data for the Effects of Oligosaccharide Treatment on LDH Release in LPS Induced Caco-2 Cells	126
B2	Raw Data for the Effect of Oligosaccharide on the Rate of Intracellular *S.* Enteritidis Clearance in U-937 Macrophages	127
B3	Raw Data for the Effects of Oligosaccharide Treatment on LDH Release in U-937 Macrophages Infected with *S*. Enteritidis	128
B4	Raw Data for the Effects of Oligosaccharide Treatment on Zebrafish Fluorescent Intensities	130
C1	Institute Animal Care and Use Committee Approval for Zebrafish Trials	131

LIST OF ABBREVIATIONS

18S	18S ribosomal RNA
ADG	average daily gain
AGP	antibiotic growth promoter
ANOVA	one-way analysis of variance
ATCC	American Type Culture Collection
BGA	brilliant green agar
Big	palm kernel cake oligosaccharides with a degree of polymerization larger than six
CFU	colony-forming unit
CTCF	corrected total cell fluorescence
DAF	diaminoflourescein
DAF-FM DA	4-amino-5-methylamino-2',7'-difluorofluorescein diacetate
DMSO	dimethyl sulfoxide
DP	degree of polymerization
dpf	days postfertilization
E. coli	*Escherichia coli*
EFB	empty fruit bunches
Elfa	elongation factor 1-alpha
ESI	electrospray ionization
EU	European Union
FDA	Food and Drug Administration
FOS	fructooligosaccharide
FOS-cont	zebrafish larvae pretreated with 250 µg/mL of FOS 24 hours before being given a combination of FOS and *S.* Enteritidis
GALT	gut-associated lymphoid tissue
GFP	green fluorescent protein
GI	gastrointestinal
GOS	galactooligosaccharides
GRAS	Generally Recognized as Safe

hpf	hours postfertilization
HPLC	high performance liquid chromatography
IACUC	Institutional Animal Care and Use Committee
IFN- ɣ	interferon-γ
IgA	immunoglobulin A
IL-11b	interleukin-11
IL-1β	interleukin-1β
IL-6	interleukin-6
IL-8	interleukin-8
iNTS	invasive non-typhoidal *Salmonella*
LC-QTOF/MS	liquid chromatography quadrupole time-of-flight mass spectrometry
LDH	lactate dehydrogenase
LPS	lipopolysaccharide
MALT	mucosa-associated lymphoid tissue
ME	metabolizable energy
MEM	minimum essential medium
MLN	mesenteric lymph nodes
MOS	mannanoligosaccharide
MPOB	Malaysian Palm Oil Board's
MPX	myeloperoxidase
Mpx	myeloperoxidase
NDOs	non-digestible oligosaccharides
NETs	neutrophil extracellular traps
NO	nitric oxide
NTS	non-typhoidal *Salmonella*
OligoPKC	palm kernel cake oligosaccharides with a degree of polymerization equal to or less than six
OligoPKC-cont	zebrafish larvae pretreated with 250 µg/mL of OligoPKC for 24 hours before being given a combination of OligoPKC and *S.* Enteritidis
PBS	phosphate buffered saline
PCR	polymerase chain reaction

PKC	palm kernel cake
PMA	phorbol 12-myristate 13-acetate
POME	palm oil mill effluent
PPF	palm pressed fiber
Pre-FOS	zebrafish larvae pretreated with 250 µg/mL of FOS for 24 hours before *S*. Enteritidis infection
Pre-OligoPKC	zebrafish larvae pretreated with 250 µg/mL of OligoPKC for 24 hours before *S*. Enteritidis infection
PTU	1-phenyl2-thiourea
qRT-PCR	quantitative real-time PCR
rcf	relative centrifugal force
RI	reflective index
RID	refractive index detector
RNA	ribonucleic acid
ROS	reactive oxygen species
RPMI	Roswell Park Memorial Institute
S. Enteritidis	*Salmonella enterica* serovar (ser.) Enteritidis
S. Typhimurium	*Salmonella enterica* serovar (ser.) Typhimurium
SCFA	short-chain fatty acids
SECtrl	zebrafish larvae inoculated with *S*. Enteritidis but no NDOs treatment
SEM	standard error of the mean
Small	palm kernel cake oligosaccharides with a degree of polymerization equal to or less than six
TAE	Tris acetate EDTA
TLR2	Toll-Like Receptor 2
TLR4	Toll-like receptor 4
TNF-α	tumour necrosis factor-α
VRI	Veterinary Research Institute

CHAPTER 1

INTRODUCTION

Animal studies have shown that non-digestible oligosaccharides (NDOs) from palm kernel cake (PKC) were capable of increasing the number of beneficial gut microbiota as well as improving serum immunoglobulin concentrations (Jahromi, Shokryazdan, Idrus, Ebrahimi, Bashokouh, et al., 2017; Rezaei et al., 2019; Rezaei et al., 2015). The production of immunoglobulins constitutes part of the adaptive immune response and functions by binding to their specific antigen, in this case pathogens, and marking it for destruction by phagocytic cells (Alberts et al., 2002). However, in very young vertebrae, the adaptive immune system has yet to be fully developed and this causes them to rely heavily on their maternal antibodies and the innate immune response for protection against pathogens (Hamal, Burgess, Pevzner, & Erf, 2006; H. Wang, Ji, Shao, & Zhang, 2012). Even so, the protective effect of maternal antibodies decreases over time and this leaves the young vertebrae vulnerable to pathogenic infections (Niewiesk, 2014). Hence this study aimed to investigate the effects of NDOs from PKC on the innate immune system against *Salmonella enterica* serovar (ser.) Enteritidis (*S*. Enteritidis) using a combination of *in vitro* and *in vivo* experiments.

PKC is a by-product of the palm oil industry. The inclusion of PKC in ruminant feed has been successful with reports stating that up to 80% of ruminant feed could be supplemented with PKC but PKC's inclusion in poultry feed was limited due to its high fiber content (Hishamuddin, 2001; Saenphoom, Liang, Ho, Loh, & Rosfarizan, 2013; Zahari & Alimon, 2004). However, recent research has shown that PKC may be a rich source of NDOs with potential prebiotic and immunomodulating activities (Jahromi et al., 2016; Jahromi, Shokryazdan, Idrus, Ebrahimi, Bashokouh, et al., 2017).

The regulation of immunity is important as the main function of the immune system is to keep pathogens from harming the balance and functions of the biological environment; i.e., the body (Alberts et al., 2002). The immune system in vertebrae can be divided into the innate and adaptive immune response. The adaptive immune response involves the recognition of specific antigens while the innate has an 'all-purpose' approach to pathogen recognition and elimination and is the focus of this study (Murphy, 2011; Raven & Johnson, 2002). Currently, there are findings indicating that NDOs are able to enhance the innate immune system. For example, both fructooligosaccharide (FOS)-inulin and β-1,4 mannobiose appear to enhance the *Salmonella* killing activity of macrophages (Babu, Sommers, Harrison, & Balan, 2012; Ibuki, Kovacs-Nolan, Fukui, Kanatani, & Mine, 2011).
However different types and sizes of oligosaccharides produce different outcomes. For example, Ito *et al.* (2011) report that fructans with lower degree of polymerization (DP) were better able to increase cecal IgA concentrations, while Badia, Lizardo, Martínez, and Brufau (2013) reports that β-galactomannan

and mannanoligosaccharide (MOS) but not D-mannose were capable of reducing *Salmonella* induced proinflammatory interleukin-6 (IL-6) and interleukin (CXCL8). Characterization of NDOs from PKC have shown that mannose makes up 45.1% to 57.3% of its monosaccharides (Bello et al., 2018; Jahromi et al., 2016). Current research shows that the immunomodulatory activity of mannose has been linked to the interactions with surface receptors of the innate immune system (Gruden-Movsesijan & Milosavljevic, 2006; Hsu et al., 2009; Kovacs-Nolan, Kanatani, Nakamura, Ibuki, & Mine, 2013). Apart from interacting with the surface receptors of the innate immune system, mannose is also capable of acting as a receptor analog to Type 1 fimbrial FimH adhesins found on pathogens such as *S*. Enteritidis (Grzymajło, Kuźmińska-Bajor, Jaworski, Dobryszycki, & Ugorski, 2010). The interaction between mannose and Type 1 fimbrial FimH adhesins causes the fimbrial proteins to agglutinate and thus preventing *S*. Enteritidis adhesion to the cells of the gastrointestinal tract (Old, 1972).

S. Enteritidis was chosen for this study because it is one of the most often isolated serovar in the poultry industry with poultry meat and its products being a staple of the Malaysian diet (Bahri, Ariffin, & Mohtar, 2019; Foley et al., 2011). The number of chickens reared in Malaysia in 2018 is estimated to be 311,978,594 and it is the largest sector of Malaysia's livestock industry ("Selected Agricultural Indicators, Malaysia, 2019," 2019). The sampling of raw chicken meat from wet markets and hypermarkets in Selangor found that *S*. Enteritidis was present in 6.7% of the meat sampled (Thung et al., 2016). In order to suppress the growth and spread of pathogens in areas of high livestock density, the application of antibiotic growth promoter (AGP) was incorporated into animal feed and it is estimated that for every kilogram of chicken meat produced 148 mg of AGP were used (H.-M. Chen, Wang, Su, & Chiu, 2013; Hughes & Heritage, 2004; Van Boeckel et al., 2015). However, this led to the emergence of antibiotic resistant pathogens and with the banning of AGP by the European Union (EU), alternatives such as prebiotics and probiotics are gaining popularity as supplements in animal nutrition (Markowiak & Śliżewska, 2018; Millet & Maertens, 2011).

Prebiotics are often NDOs such as FOS and galactooligosaccharides (GOS) that have been proven to not only resist host digestion but are also able to selectively influence the composition and activity of microbes found within the gut to the benefit of the host (Roberfroid et al., 2010). The Food and Drug Administration (FDA) states that the consumption of FOS up to 20 g/day is safe for the general population while for infants under one year of age, the addition of 4.2 g/day of FOS into infant food is permissible ("Determination of the generally recognized as safe (GRAS) status of fructooligosaccharides as food ingredient ", 2016). Studies have reported that the supplementation of prebiotics during the transitional phase from liquid food to solids in infants and toddlers led to better gut microbiota, fewer gastrointestinal infections and better gut barrier integrity (McKeen et al., 2019; Veereman, 2007). In *Salmonella* challenged chicks, the supplementation of NDOs showed a decreased in *Salmonella enterica* serovar (ser.) Typhimurium (*S*. Typhimurium) colonization (Rezaei et al., 2019).

In vertebrae, the gut mucosal immune system is considered the largest immune organ of the body and it is affected by a wide range of variables such as the composition of the individual's gut microbiota, the subject's age, genetics, diet, nutritional status and lifestyle (Binns, 2013; Shi, Li, Duan, & Niu, 2017). In order to minimize these variables, an *in vitro S*. Enteritidis infection model utilizing Caco-2 epithelial cells and U-937 macrophages will be used in this study. Epithelial and macrophage cells were chosen for the *in vitro* study because the epithelial lining of the gastrointestinal tract functions as the first line of defense against pathogens by providing a physical barrier to minimize contact between the intestinal microbiota and the host's biological environment (Hammer, Morris, Earley, & Choudhry, 2015). Macrophages on the other hand were chosen because as part of the innate immune response, macrophages are broadly specific and express several surface receptors that are able to respond to carbohydrates (Murphy, 2011; Raven & Johnson, 2002). Apart from that, *in vitro* studies have also shown that NDOs are capable of reducing *Salmonella* adhesion to Caco-2 cells and improve the *Salmonella* killing activity of macrophages (Coppa et al., 2006; Ibuki et al., 2011).

While *in vitro* studies allows for better reproducibility and control of variables, it lacks the complexity and interactions of difference cells in the physiological environment (Chanput, Peters, & Wichers, 2015). In this study, zebrafish (*Danio rerio*) larvae were used as an *in vivo S*. Enteritidis infection model. Zebrafish was chosen as an animal model to study the innate immune system because the adaptive immune system of zebrafishes are not fully developed until they have reached two to four weeks of age and these fishes are often used as an animal models to study the innate immune system of vertebrae (Meijer & Spaink, 2011; Renshaw & Trede, 2012). In addition, zebrafish larvae up to five days postfertilization (dpf) are optically transparent and this transparency may be extended with the use of 1-phenyl2-thiourea (PTU) during embryogenesis (Chávez, Aedo, Fierro, Allende, & Egaña, 2016; Karlsson, von Hofsten, & Olsson, 2001). The optical transparency of zebrafish larvae provides an advantage over other animal models as it enables us to carry out non-invasive imaging studies (Harvie & Huttenlocher, 2015); such as the observation of anti-inflammatory effects of NDOs *in vivo* using nitric oxide (NO) as a marker (Lepiller et al., 2007) as well as the monitoring of the colonization patterns (Varas et al., 2017) of *S*. Enteritidis within the zebrafishes' gastrointestinal tract with and without NDOs treatment.

Other advantages which prompted the use of the zebrafish infection model over other existing animal models has been attributed to its increasing use as a model for human diseases (C. Xu & Zon, 2010). This is because zebrafishes have been reported to possess at least 70% of the genes found in humans (Howe et al., 2013) and share similarity with the organ system of humans (Goldsmith & Jobin, 2012). Coupled with its high reproduction rate, ease of handling and low cost (Goldsmith & Jobin, 2012; F. R. Khan & Alhewairini, 2018), this allows for the high-throughput screening of various therapeutics (Mathias, Saxena, & Mumm, 2012); in this case the utilization of NDOs in a *S*. Enteritidis infection model.

1.1 Problem statement and justification

The use of AGP in the livestock industry has been linked to the emergence of antibiotic resistant pathogens (Millet & Maertens, 2011) and with the EU wide ban on the use of AGP in 2006, research into alternatives to the use of AGP has gained traction (Maron, Smith, & Nachman, 2013; Millet & Maertens, 2011). PKC is a by-product of the palm oil industry with limited applicability as a feed ingredient in the poultry industry due to its high fiber content (Saenphoom et al., 2013). Previous studies have shown that oligosaccharides from PKC possess prebiotic potential and its inclusion in the diet of young chickens appear to confer a protective effect against *Salmonella* colonization (Rezaei et al., 2019). However, there are several pathways through which NDOs from PKC may exert their prebiotic properties (Hoyles & Vulevic, 2008; Shoaf-Sweeney & Hutkins, 2008). As the immune response in young vertebrae is often immature (Simon, Hollander, & McMichael, 2015), this study focuses on the immunomodulatory effects; that is, the ability of a tested compound to modify the immune response (Gea-Banacloche, 2006) of NDOs from PKC on the innate immune system using a combination of *in vitro* and *in vivo* approaches.

1.2 Hypothesis

It is hypothesized that NDOs from PKC reduces *S*. Enteritidis adherence to the epithelial cells of the gastrointestinal tract, improve the rate of pathogen clearance in macrophages, reduce cellular damage and inflammation, and alter the expression of genes in order the alleviate the symptoms of salmonellosis.

1.3 Objectives

The main objective of this study was to evaluate the immunomodulatory effects of NDOs from PKC on the innate immune system in a *S*. Enteritidis infection model utilizing *in vitro* and *in vivo* approaches. To achieve this end, the following specific objectives were set.

The specific objectives were:

i) to extract and prepare low molecular weight non-digestible oligosaccharide from PKC and to evaluate its ability to reduce *S*. Enteritidis adherence to Caco-2 cells; to increase the rate of *S*. Enteritidis clearance in U-937 macrophages and; to reduce cellular damage caused by *S*. Enteritidis.

ii) to examine whether the PKC oligosaccharides can reduce lipopolysaccharide (LPS) induced inflammation through the use of NO as a marker in zebrafish larvae.

iii) to determine the effects of PKC oligosaccharides on the colonization patterns of *S*. Enteritidis within the zebrafish larvae's gastrointestinal tract and its effects on the zebrafish larvae's gene expression.

REFERENCES

Aachary, A. A., & Prapulla, S. G. (2011). Xylooligosaccharides (XOS) as an emerging prebiotic: microbial synthesis, utilization, structural characterization, bioactive properties, and applications. *Compr Rev Food Sci Food Saf, 10*(1), 2-16.

Akbari, P., Fink-Gremmels, J., Willems, R. H., Difilippo, E., Schols, H. A., Schoterman, M. H., . . . Braber, S. (2017). Characterizing microbiota-independent effects of oligosaccharides on intestinal epithelial cells: Insight into the role of structure and size. *Eur J Nutr, 56*(5), 1919-1930.

Akil, L., Ahmad, H. A., & Reddy, R. S. (2014). Effects of climate change on Salmonella infections. *Foodborne Pathog Dis, 11*(12), 974-980.

Akpinar, O., Erdogan, K., & Bostanci, S. (2009). Enzymatic production of xylooligosaccharide from selected agricultural wastes. *Food Bioprod Process, 87*(2), 145-151.

Al-Manhel, A., & Niamah, A. (2017). Mannan extract from Saccharomyces cerevisiae used as prebiotic in bioyogurt production from buffalo milk. *Int Food Res J, 24*(5).

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). The adaptive immune system. *Mol Biol Cell, 4*, 1363-1402.

Aleström, P., D'Angelo, L., Midtlyng, P. J., Schorderet, D. F., Schulte-Merker, S., Sohm, F., & Warner, S. (2020). Zebrafish: Housing and husbandry recommendations. *Lab Anim, 54*(3), 213-224.

Allen, V., Fernandez, F., & Hinton, M. (1997). Evaluation of the influence of supplementing the diet with mannose or palm kernel meal on salmonella colonisation in poultry. *Br. Poult. Sci., 38*(5), 485-488.

Altekruse, S. F., Bauer, N., Chanlongbutra, A., DeSagun, R., Naugle, A., Schlosser, W., . . . White, P. (2006). *Salmonella* Enteritidis in broiler chickens, United States, 2000–2005. *Emerg Infect Dis, 12*(12), 1848.

Anadón, A., Martínez-Larrañaga, M. R., Ares, I., & Martínez, M. A. (2016). Prebiotics: safety and toxicity considerations *Nutraceuticals* (pp. 757-775): Elsevier.

Andersen, C. L., Jensen, J. L., & Ørntoft, T. F. (2004). Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. *Cancer Res, 64*(15), 5245-5250.

Animal Model. (n.d.). Retrieved from https://www.genome.gov/genetics-glossary/Animal-

Model#:~:text=An%20animal%20model%20is%20a,prevention%2C%20diagnosis%2C%20and%20treatment.

Avdagić, N., Zaćiragić, A., Babić, N., Hukić, M., Šeremet, M., Lepara, O., & Nakaš-Ićindić, E. (2013). Nitric oxide as a potential biomarker in inflammatory bowel disease. *Bosn J Basic Med Sci, 13*(1), 5.

Babu, U. S., Sommers, K., Harrison, L. M., & Balan, K. V. (2012). Effects of fructooligosaccharide-inulin on Salmonella-killing and inflammatory gene expression in chicken macrophages. *Vet. Immunol. Immunopathol., 149*(1-2), 92-96.

Badia, R., Lizardo, R., Martínez, P., & Brufau, J. (2013). Oligosaccharide structure determines prebiotic role of β-galactomannan against *Salmonella enterica* ser. Typhimurium *in vitro*. *Gut microbes, 4*(1), 72-75.

Bahri, S., Ariffin, A., & Mohtar, S. (2019). Critical review on food security in Malaysia for broiler industry. *Int J Acad Res Bus Soc Sci, 9*(7), 869-876.

Balligand, J.-L., & Cannon, P. J. (1997). Nitric oxide synthases and cardiac muscle: autocrine and paracrine influences. *Arterioscler Thromb Vasc Biol, 17*(10), 1846-1858.

Ballou, C. E. (1970). A study of the immunochemistry of three yeast mannans. *J Biol Chem., 245*(5), 1197-1203.

Banerjee, S., & Leptin, M. (2014). Systemic response to ultraviolet radiation involves induction of leukocytic il-1β and inflammation in zebrafish. *J Immunol, 193*(3), 1408-1415.

Banerjee, S., & Mazumdar, S. (2012). Electrospray ionization mass spectrometry: a technique to access the information beyond the molecular weight of the analyte. *Int. J. Anal. Chem., 2012*.

Basha, S., Surendran, N., & Pichichero, M. (2014). Immune responses in neonates. *Expert Rev Clin Immunol, 10*(9), 1171-1184.

Basu, S. K., Thomas, J. E., & Acharya, S. N. (2007). Prospects for growth in global nutraceutical and functional food markets: a Canadian perspective. *Aust. J. Basic Appl. Sci, 1*(4), 637-649.

Bäumler, A. J., Hargis, B. M., & Tsolis, R. M. (2000). Tracing the origins of Salmonella outbreaks. *Science, 287*(5450), 50-52.

Baurhoo, B., Phillip, L., & Ruiz-Feria, C. (2007). Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. *Poult Sci, 86*(6), 1070-1078.

Beeching, N., Jones, R., & Gazzard, B. (2011). 4 Gastrointestinal opportunistic infections. *HIV Med, 12*(s2), 43-54.

Bello, B., Mustafa, S., Tan, J. S., Ibrahim, T. A. T., Tam, Y. J., Ariff, A. B., . . . Abbasiliasi, S. (2018). Evaluation of the effect of soluble polysaccharides of palm kernel cake as a potential prebiotic on the growth of probiotics. *3 Biotech, 8*(8), 346.

Ben-Sasson, S. Z., Hu-Li, J., Quiel, J., Cauchetaux, S., Ratner, M., Shapira, I., . . . Paul, W. E. (2009). IL-1 acts directly on CD4 T cells to enhance their antigen-driven expansion and differentiation. *Proc Natl Acad Sci U S A, 106*(17), 7119-7124.

Benard, E. L., van der Sar, A. M., Ellett, F., Lieschke, G. J., Spaink, H. P., & Meijer, A. H. (2012). Infection of zebrafish embryos with intracellular bacterial pathogens. *J Vis Exp*(61), e3781-e3781.

Bermudez-Brito, M., Plaza-Díaz, J., Fontana, L., Munoz-Quezada, S., & Gil, A. (2013). In vitro cell and tissue models for studying host–microbe interactions: a review. *Br J Nutr, 109*(S2), S27-S34.

Berri, M., Hogan, D., Saade, G., Roche, S., Velge, P., Virlogeux-Payant, I., & Meurens, F. (2020). IPEC-1 variable immune response to different serovars of *Salmonella enterica* subsp. enterica. *Vet Immunol Immunopathol, 220*, 109989.

Bickel, M. (1993). The role of interleukin-8 in inflammation and mechanisms of regulation. *Journal of periodontology, 64*(5 Suppl), 456-460.

Biggs, P., Parsons, C., & Fahey, G. (2007). The effects of several oligosaccharides on growth performance, nutrient digestibilities, and cecal microbial populations in young chicks. *Poult. Sci., 86*(11), 2327-2336.

Binns, N. (2013). Probiotics, prebiotics and the gut microbiota. *Probiotics, prebiotics and the gut microbiota*.

Bishop, J. L., Arena, E. T., Harder, K. W., & Finlay, B. B. (2011). Anti-*Salmonella* Immunity: Highlighting New Research in Vaccines, Mucosal Immunology and Systemic Disease. In S. Porwollik (Ed.), *Salmonella: From Genome to Function* (pp. 241). Norfolk: Caister Academic Press.

Bonardi, S. (2017). Salmonella in the pork production chain and its impact on human health in the European Union. *Epidemiol Infect, 145*(8), 1513-1526.

Bonneaud, C., Mazuc, J., Gonzalez, G., Haussy, C., Chastel, O., Faivre, B., & Sorci, G. (2003). Assessing the cost of mounting an immune response. *Am Nat, 161*(3), 367-379.

Bouwhuis, M., McDonnell, M., Sweeney, T., Mukhopadhya, A., O'Shea, C., & O'Doherty, J. (2017). Seaweed extracts and galacto-oligosaccharides improve intestinal health in pigs following Salmonella Typhimurium challenge. *animal, 11*(9), 1488-1496.

Bozkurt, M., Küçükyilmaz, K., Çatli, A., & Çinar, M. (2008). Growth performance and slaughter characteristics of broiler chickens fed with antibiotic, mannan oligosaccharide and dextran oligosaccharide supplemented diets. *Int. J. Poult. Sci, 7*(10), 969-977.

Brandl, K., & Schnabl, B. (2015). Is intestinal inflammation linking dysbiosis to gut barrier dysfunction during liver disease? *Expert Rev Gastroenterol Hepatol, 9*(8), 1069-1076.

Browne, A. J., Hamadani, B. H. K., Kumaran, E. A., Rao, P., Longbottom, J., Harriss, E., . . . Baker, S. (2020). Drug-resistant enteric fever worldwide, 1990 to 2018: a systematic review and meta-analysis. *BMC Med, 18*(1), 1.

Bunning, S., & De Pauw, E. (2017). Land resource planning for sustainable land management. *Land and Water Division Working Paper (FAO) eng no. 14*.

Campbell, C., Su, T., Lau, R. P., Shah, A., Laurie, P. C., Avalos, B., . . . Stachura, D. L. (2015). Zebrafish embryonic stromal trunk (ZEST) cells support hematopoietic stem and progenitor cell (HSPC) proliferation, survival, and differentiation. *Exp Hematol, 43*(12), 1047-1061.

Capitán-Cañadas, F., Ortega-González, M., Guadix, E., Zarzuelo, A., Suárez, M. D., de Medina, F. S., & Martínez-Augustin, O. (2014). Prebiotic oligosaccharides directly modulate proinflammatory cytokine production in monocytes via activation of TLR 4. *Mol Nutr Food Res, 58*(5), 1098-1110.

Capper, J., Berger, L., Brashears, M., & Jensen, H. (2013). Animal feed vs. human food: Challenges and opportunities in sustaining animal agriculture toward 2050.

Cavaillon, J.-M., & Adib-Conquy, M. (2002). The pro-inflammatory cytokine cascade *Immune Response in the Critically Ill* (pp. 37-66). Germany: Springer.

Cerveró, J. M., Skovgaard, P. A., Felby, C., Sørensen, H. R., & Jørgensen, H. (2010). Enzymatic hydrolysis and fermentation of palm kernel press cake for production of bioethanol. *Enzyme Microb Technol, 46*(3), 177-184.

Chandrasekaran, M. (2015). *Enzymes in Food and Beverage Processing*: CRC Press.

Chang, A. Y., Chau, V., Landas, J. A., & Pang, Y. (2017). Preparation of calcium competent *Escherichia coli* and heat-shock transformation. *JEMI Methods, 1*, 22-25.

Chanput, W., Peters, V., & Wichers, H. (2015). THP-1 and U937 Cells. In K. Verhoeckx, P. Cotter, I. López-Expósito, C. Kleiveland, T. Lea, A. Mackie, T. Requena, D. Swiatecka, & H. Wichers (Eds.), *The Impact of Food Bioactives on Health: in vitro and ex vivo models* (pp. 147-159). Cham: Springer International Publishing.

Chantret, I., Barbat, A., Dussaulx, E., Brattain, M. G., & Zweibaum, A. (1988). Epithelial polarity, villin expression, and enterocytic differentiation of cultured human colon carcinoma cells: a survey of twenty cell lines. *Cancer Res, 48*(7), 1936-1942.

Charalampopoulos, D., & Rastall, R. A. (2009). *Prebiotics and Probiotics Science and Technology*: Springer.

Chaudhry, H., Zhou, J., Zhong, Y., Ali, M. M., McGuire, F., Nagarkatti, P. S., & Nagarkatti, M. (2013). Role of cytokines as a double-edged sword in sepsis. *In Vivo, 27*(6), 669-684.

Chávez, M. N., Aedo, G., Fierro, F. A., Allende, M. L., & Egaña, J. T. (2016). Zebrafish as an emerging model organism to study angiogenesis in development and regeneration. *Front Physiol, 7*.

Chen, H.-M., Wang, Y., Su, L.-H., & Chiu, C.-H. (2013). Nontyphoid Salmonella infection: microbiology, clinical features, and antimicrobial therapy. *Pediatr Neonatol, 54*(3), 147-152.

Chen, J., Liang, R.-h., Liu, W., Li, T., Liu, C.-m., Wu, S.-s., & Wang, Z.-j. (2013). Pectic-oligosaccharides prepared by dynamic high-pressure microfluidization and their *in vitro* fermentation properties. *Carbohydr Polym, 91*(1), 175-182.

Chen, T., Kim, C. Y., Kaur, A., Lamothe, L., Shaikh, M., Keshavarzian, A., & Hamaker, B. R. (2017). Dietary fibre-based SCFA mixtures promote both protection and repair of intestinal epithelial barrier function in a Caco-2 cell model. *Food Funct, 8*(3), 1166-1173.

Chen, W., Jahromi, M., Candyrine, S., Liang, J., Abdullah, N., & Idrus, Z. (2018). Enzymatic hydrolysis drastically reduces fibre content of palm-kernel expeller, but without enhancing performance in broiler chickens. *Anim Prod Sci*.

Chen, W. L., Liang, J. B., Jahromi, M. F., Abdullah, N., Ho, Y. W., & Tufarelli, V. (2014). Enzyme Treatment Enhances Release of Prebiotic Oligosaccharides from Palm Kernel Expeller. *BioResources, 10*(1), 196-209.

Choi, H. W., Brooking, R., Neupane, S., Lee, C.-J., Miao, E., Staats, H. F., & Abraham, S. N. (2014). Salmonella Typhimurium Impedes Innate Immunity With a Mast Cell-Suppressing Tyrosine Phosphatase Sptp. *J Allergy Clin Immunol, 133*(2), AB247.

Clegg, S., & Gerlach, G. (1987). Enterobacterial fimbriae. *J Bacteriol, 169*(3), 934.

Clench, M. H., & Mathias, J. R. (1995). The avian cecum: a review. *Wilson Bull*, 93-121.

Coppa, G. V., Zampini, L., Galeazzi, T., Facinelli, B., Ferrante, L., Capretti, R., & Orazio, G. (2006). Human milk oligosaccharides inhibit the adhesion to Caco-2 cells of diarrheal pathogens: *Escherichia coli, Vibrio cholerae*, and *Salmonella* fyris. *Pediatr. Res., 59*(3), 377-382.

Corzo-Martínez, M., García-Campos, G., Montilla, A., & Moreno, F. J. (2016). Tofu whey permeate is an efficient source to enzymatically produce prebiotic fructooligosaccharides and novel fructosylated α-galactosides. *J Agric Food Chem, 64*(21), 4346-4352.

Cunningham, M., Azcarate-Peril, M. A., Barnard, A., Benoit, V., Grimaldi, R., Guyonnet, D., . . . Obis, D. (2021). Shaping the future of probiotics and prebiotics. *Trends Microbiol*.

Dal, N. J. K., Kocere, A., Wohlmann, J., Van Herck, S., Bauer, T. A., Resseguier, J., . . . De Geest, B. G. (2020). Zebrafish embryos allow prediction of nanoparticle circulation times in mice and facilitate quantification of nanoparticle–cell interactions. *Small, 16*(5), 1906719.

das Graças Vaz-Tostes, M., Viana, M. L., Grancieri, M., dos Santos Luz, T. C., de Paula, H., Pedrosa, R. G., & Costa, N. M. B. (2014). Yacon effects in immune response and nutritional status of iron and zinc in preschool children. *Nutrition, 30*(6), 666-672.

De Vuyst, L., & Leroy, F. (2007). Bacteriocins from lactic acid bacteria: production, purification, and food applications. *J Mol Microbiol Biotechnol, 13*(4), 194-199.

DeGruttola, A. K., Low, D., Mizoguchi, A., & Mizoguchi, E. (2016). Current understanding of dysbiosis in disease in human and animal models. *Inflammatory Bowel Dis., 22*(5), 1137-1150.

Delzenne, N. M., & Roberfroid, M. (1994). Physiological effects of non-digestible oligosaccharides. *LWT-Food Sci Technol, 27*(1), 1-6.

Derrien, M., & van Hylckama Vlieg, J. E. (2015). Fate, activity, and impact of ingested bacteria within the human gut microbiota. *Trends Microbiol., 23*(6), 354-366.

Des Gachons, C. P., & Breslin, P. A. (2016). Salivary amylase: digestion and metabolic syndrome. *Curr Diab Rep, 16*(10), 102.

Determination of the generally recognized as safe (gras) status of fructooligosaccharides as food ingredient (2016). (GRAS Notice 623: Fructooligosaccharides). Retrieved 29 November 2019, from FDA https://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&sort=GRN_No&order=DESC&startrow=1&type=basic&search=623

Dhingra, D., Michael, M., Rajput, H., & Patil, R. (2012). Dietary fibre in foods: a review. *J Food Sci Technol, 49*(3), 255-266.

Djeffal, S., Mamache, B., Elgroud, R., Hireche, S., & Bouaziz, O. (2018). Prevalence and risk factors for Salmonella spp. contamination in broiler chicken farms and slaughterhouses in the northeast of Algeria. *Vet World, 11*(8), 1102.

Dua Pelajar Keracunan Makanan Dibenarkan Keluar Hospital. (2014). Retrieved 15 March 2015, from Bernama http://web6.bernama.com/bernama//v3/bm/news_lite.php?id=1015540

Egun, N. K. (2012). The Waste to Wealth Concept; Waste Market Operation in Delta state Nigeria. *Greener J Soc Sci, 2*(6), 206-212.

Ekman, P., Saarinen, M., He, Q., Virtala, M., Salmi, M., & Granfors, K. (1999). Human monocytic U937 cells kill *Salmonella in vitro* by NO-independent mechanisms. *Infect Immun, 67*(7), 3670-3673.

The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. (2018). *EFSA J, 16*(12), e05500.

Feasey, N. A., Dougan, G., Kingsley, R. A., Heyderman, R. S., & Gordon, M. A. (2012). Invasive non-typhoidal salmonella disease: an emerging and neglected tropical disease in Africa. *Lancet, 379*(9835), 2489-2499.

Fent, K., Weisbrod, C. J., Wirth-Heller, A., & Pieles, U. (2010). Assessment of uptake and toxicity of fluorescent silica nanoparticles in zebrafish (*Danio rerio*) early life stages. *Aquat Toxicol, 100*(2), 218-228.

Ferrari, R. G., Rosario, D. K., Cunha-Neto, A., Mano, S. B., Figueiredo, E. E., & Conte-Junior, C. A. (2019). Worldwide epidemiology of *Salmonella* serovars in animal-based foods: a meta-analysis. *Appl Environ Microbiol, 85*(14), e00591-00519.

Figueiredo, R. T., Carneiro, L. A., & Bozza, M. T. (2011). Fungal surface and innate immune recognition of filamentous fungi. *Front Microbiol, 2*, 248.

Fisher, R. A., Cheverton, A. M., & Helaine, S. (2016). *Analysis of Macrophage-Induced Salmonella Persisters*. Berlin/Heidelberg, Germany: Springer.

Fisher, R. A., Gollan, B., & Helaine, S. (2017). Persistent bacterial infections and persister cells. *Nat Rev Microbiol, 15*(8), 453.

Flesch, M., Kilter, H., Cremers, B., Laufs, U., Südkamp, M., Ortmann, M., . . . Böhm, M. (1999). Effects of endotoxin on human myocardial contractility involvement of nitric oxide and peroxynitrite. *J Am Coll Cardiol, 33*(4), 1062-1070.

Flint, H. J., Scott, K. P., Duncan, S. H., Louis, P., & Forano, E. (2012). Microbial degradation of complex carbohydrates in the gut. *Gut microbes, 3*(4), 289-306.

Foley, S. L., Nayak, R., Hanning, I. B., Johnson, T. J., Han, J., & Ricke, S. C. (2011). Population dynamics of Salmonella enterica serotypes in commercial egg and poultry production. *Appl Environ Microbiol, 77*(13), 4273-4279.

Franco, D. M., Arevalo-Rodriguez, I., i Figuls, M. R., & Zamora, J. (2015). Interleukin-6 for diagnosis of sepsis in critically ill adult patients. *Cochrane Libr*(7).

Freidl, G., Schoss, S., Te Wierik, M., Heck, M., Tolsma, P., Urbanus, A., . . . Friesema, I. (2018). Tracing Back the Source of an Outbreak of Salmonella Typhimurium; National Outbreak Linked to the Consumption of Raw and Undercooked Beef Products, the Netherlands, October to December 2015. *PLoS Curr, 10*.

Friedman, A. (2008). Oral tolerance in birds and mammals: digestive tract development determines the strategy. *J Appl Poult Res, 17*(1), 168-173.

Gal-Mor, O., Boyle, E. C., & Grassl, G. A. (2014). Same species, different diseases: how and why typhoidal and non-typhoidal Salmonella enterica serovars differ. *Front Microbiol, 5*, 391.

Gazzinelli, R. T., Oswald, I., James, S., & Sher, A. (1992). IL-10 inhibits parasite killing and nitrogen oxide production by IFN-gamma-activated macrophages. *J Immunol, 148*(6), 1792-1796.

Gea-Banacloche, J. C. (2006). Immunomodulation *Principles of molecular medicine* (pp. 893-904): Humana Press.

Geier, M., Torok, V., Allison, G., Ophel-Keller, K., & Hughes, R. (2009). Indigestible carbohydrates alter the intestinal microbiota but do not influence the performance of broiler chickens. *J Appl Microbiol, 106*(5), 1540-1548.

Georgieva, R., Yocheva, L., Tserovska, L., Zhelezova, G., Stefanova, N., Atanasova, A., . . . Rumyan, N. (2015). Antimicrobial activity and antibiotic susceptibility of Lactobacillus and Bifidobacterium spp.

intended for use as starter and probiotic cultures. *Biotechnol Biotechnol Equip, 29*(1), 84-91.

Ghasemi, A., Asl, S. Z., Mehrabi, Y., Saadat, N., & Azizi, F. (2008). Serum nitric oxide metabolite levels in a general healthy population: relation to sex and age. *Life Sci, 83*(9-10), 326-331.

Gibson, G. R., Probert, H. M., Van Loo, J., Rastall, R. A., & Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutr Res Rev, 17*(2), 259-275.

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonie microbiota: introducing the concept of prebiotics. *J Nutr, 125*, 1401-1412.

Gibson, G. R., Scott, K. P., Rastall, R. A., Tuohy, K. M., Hotchkiss, A., Dubert-Ferrandon, A., . . . Loh, G. (2010). Dietary prebiotics: current status and new definition. *Food Sci Tech Bull Funct Foods*(7), 1-19.

Goggins, M. G., Shah, S. A., Goh, J., Cherukuri, A., Weir, D. G., Kelleher, D., & Mahmud, N. (2001). Increased urinary nitrite, a marker of nitric oxide, in active inflammatory bowel disease. *Mediators Inflamm, 10*(2), 69-73.

Goldsmith, J. R., & Jobin, C. (2012). Think small: zebrafish as a model system of human pathology. *Journal of Biomedicine and Biotechnology, 2012*.

Gómez, B. n., Gullón, B., Remoroza, C., Schols, H. A., Parajó, J. C., & Alonso, J. L. (2014). Purification, Characterization, and Prebiotic Properties of Pectic Oligosaccharides from Orange Peel Wastes. *J Agric Food Chem, 62*(40), 9769-9782.

Gorbach, S. (1996). Chpter 95: Microbiology of the Gastrointestinal Tract. *Medical Microbiology, 4th ed.(Baron, S. ed.), University of Texas Medical Branch at Galveston, Galveston. Retrieved from http://www. ncbi. nlm. nih. gov/books/NBK7670/# _ncbi_dlg_citbx_NBK7670*.

Greenwood-Van Meerveld, B., Tyler, K., & Keith, J. C. (2000). Recombinant human interleukin-11 modulates ion transport and mucosal inflammation in the small intestine and colon. *Lab Invest, 80*(8), 1269-1280.

Grieshop, C., Flickinger, E., Bruce, K., Patil, A., Czarnecki-Maulden, G., & Fahey Jr, G. (2004). Gastrointestinal and immunological responses of senior dogs to chicory and mannan-oligosaccharides. *Arch Anim Nutr., 58*(6), 483-494.

Growing at a Slower Pace, World Population is Expected to Reach 9.7 Billion in 2050 and Could Peak at Nearly 11 Billion around 2100. (2019). [Press release]. Retrieved from https://www.un.org/development/desa/en/news/population/world-population-prospects-2019.html

Gruden-Movsesijan, A., & Milosavljevic, L. S. (2006). The involvement of the macrophage mannose receptor in the innate immune response to infection with parasite Trichinella spiralis. *Vet Immunol Immunopathol, 109*(1-2), 57-67.

Grzymajło, K., Kuźmińska-Bajor, M., Jaworski, J., Dobryszycki, P., & Ugorski, M. (2010). The high-adhesive properties of the FimH adhesin of Salmonella enterica serovar Enteritidis are determined by a single F118S substitution. *Microbiol., 156*(6), 1738-1748.

Guo, X., Ji, C., Du, X., Ren, J., Zu, Y., Li, W., & Zhang, Q. (2019). Comparison of gene expression responses of zebrafish larvae to Vibrio parahaemolyticus infection by static immersion and caudal vein microinjection. *Aquac Fish*.

Gutierrez, R. M. P., Flores, J. M. M., & Gonzalez, A. M. N. (2018). Anti-inflammatory effect of procumbenoside B from Justicia spicigera on lipopolysaccharide-stimulated RAW 264.7 macrophages and zebrafish model. *Pharmacognosy Res, 10*(2), 218.

Meijer, A.H., & Spaink, H. P. (2011). Host-pathogen interactions made transparent with the zebrafish model. *Curr Drug Targets, 12*(7), 1000-1017.

Hajati, H., Hassanabadi, A., & Yansari, A. T. (2014). The Effect of Dietary Supplementation of Prebiotic and Probiotic on Performance, Humoral Immunity Responses and Egg Hatchability in Broiler Breeders. *Poult Sci J, 2*.

Hallstrom, K., & McCormick, B. A. (2011). Salmonella interaction with and passage through the intestinal mucosa: through the lens of the organism. *Front Microbiol, 2*, 88.

Hamal, K. R., Burgess, S. C., Pevzner, I., & Erf, G. (2006). Maternal antibody transfer from dams to their egg yolks, egg whites, and chicks in meat lines of chickens. *Poult Sci, 85*(8), 1364-1372.

Hammer, A. M., Morris, N. L., Earley, Z. M., & Choudhry, M. A. (2015). The first line of defense: the effects of alcohol on post-burn intestinal barrier, immune cells, and microbiome. *Alcohol Res: Curr Rev, 37*(2), 209.

Hardin, G. (1960). The competitive exclusion principle. *Science, 131*(3409), 1292-1297.

Hardy, H., Harris, J., Lyon, E., Beal, J., & Foey, A. D. (2013). Probiotics, prebiotics and immunomodulation of gut mucosal defences: homeostasis and immunopathology. *Nutrients, 5*(6), 1869-1912.

Harvie, E. A., & Huttenlocher, A. (2015). Non-invasive imaging of the innate immune response in a zebrafish larval model of *Streptococcus iniae* infection. *JoVE (Journal of Visualized Experiments)*(98), e52788.

Hassan, M. A. (2008). *Waste-to-wealth Through Biotechnology: For Profit, People and Planet*: Penerbit Universiti Putra Malaysia.

Henanjunda. (2017). Factory direct food grade [mannan oligosaccharides] quality mannan oligosaccharide purity 99% large quantity favorably. Retrieved from https://detail.1688.com/offer/45031592549.html

Hendriksen, R. S., Vieira, A. R., Karlsmose, S., Lo Fo Wong, D. M., Jensen, A. B., Wegener, H. C., & Aarestrup, F. M. (2011). Global monitoring of Salmonella serovar distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: results of quality assured laboratories from 2001 to 2007. *Foodborne Pathog. Dis., 8*(8), 887-900.

Hernandez, R. E., Galitan, L., Cameron, J., Goodwin, N., & Ramakrishnan, L. (2018). Delay of initial feeding of zebrafish larvae until 8 days postfertilization has no impact on survival or growth through the juvenile stage. *Zebrafish, 15*(5), 515-518.

Hibbing, M. E., Fuqua, C., Parsek, M. R., & Peterson, S. B. (2010). Bacterial competition: surviving and thriving in the microbial jungle. *Nat Rev Microbiol, 8*(1), 15.

Hickman, D., Johnson, J., Vemulapalli, T., Crisler, J., & Shepherd, R. (2017). *Commonly Used Animal Models*. UK: Elsevier.

Higginson, E. E., Simon, R., & Tennant, S. M. (2016). Animal models for salmonellosis: applications in vaccine research. *Clin Vaccine Immunol, 23*(9), 746-756.

Hildemann, W. (1962). Immunogenetic studies of poikilothermic animals. *Am Nat, 96*(889), 195-204.

Hishamuddin, M. A. (2001). Malaysian Palm Kernel Cake as Animal Feed. *Palm Oil Dev*(34), 4-6.

Hoseinifar, S. H., Soleimani, N., & Ringø, E. (2014). Effects of dietary fructo-oligosaccharide supplementation on the growth performance, haemato-immunological parameters, gut microbiota and stress resistance of common carp (Cyprinus carpio) fry. *Br J Nutr, 112*(8), 1296-1302.

Hosono, A., Ozawa, A., Kato, R., OHNISHI, Y., Nakanishi, Y., Kimura, T., & Nakamura, R. (2003). Dietary fructooligosaccharides induce immunoregulation of intestinal IgA secretion by murine Peyer's patch cells. *Biosci Biotechnol Biochem, 67*(4), 758-764.

Hou, Y., Ding, X., & Hou, W. (2015). Composition and antioxidant activity of water-soluble oligosaccharides from Hericium erinaceus. *Mol Med Rep., 11*(5), 3794-3799.

Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., . . . Matthews, L. (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature, 496*(7446), 498-503.

Howlader, D. R., Sinha, R., Nag, D., Majumder, N., Mukherjee, P., Bhaumik, U., . . . Koley, H. (2016). Zebrafish as a novel model for non-typhoidal *Salmonella* pathogenesis, transmission and vaccine efficacy. *Vaccine, 34*(42), 5099-5106.

Hoyles, L., & Vulevic, J. (2008). Diet, immunity and functional foods *GI Microbiota and Regulation of the Immune System* (pp. 79-92). TX, USA: Springer.

Hsu, T.-L., Cheng, S.-C., Yang, W.-B., Chin, S.-W., Chen, B.-H., Huang, M.-T., . . . Wong, C.-H. (2009). Profiling carbohydrate-receptor interaction with recombinant innate immunity receptor-Fc fusion proteins. *J Biol Chem., 284*(50), 34479-34489.

Hudu, S. A., Alshrari, A. S., Syahida, A., & Sekawi, Z. (2016). Cell culture, technology: enhancing the culture of diagnosing human diseases. *J Clin Diagn Res, 10*(3), DE01.

Hughes, P., & Heritage, J. (2004). Antibiotic growth-promoters in food animals. *FAO Animal Production and Health Paper*, 129-152.

Hurst, N. R., Kendig, D. M., Murthy, K. S., & Grider, J. R. (2014). The short chain fatty acids, butyrate and propionate, have differential effects on the motility of the guinea pig colon. *Neurogastroenterol Motil, 26*(11), 1586-1596.

Ibuki, M., Kovacs-Nolan, J., Fukui, K., Kanatani, H., & Mine, Y. (2011). β 1-4 mannobiose enhances *Salmonella*-killing activity and activates innate immune responses in chicken macrophages. *Vet. Immunol. Immunopathol., 139*(2), 289-295.

Idriss, H. T., & Naismith, J. H. (2000). TNFα and the TNF receptor superfamily: Structure-function relationship (s). *Microscopy research and technique, 50*(3), 184-195.

Ito, H., Takemura, N., Sonoyama, K., Kawagishi, H., Topping, D. L., Conlon, M. A., & Morita, T. (2011). Degree of polymerization of inulin-type fructans differentially affects number of lactic acid bacteria, intestinal immune functions, and immunoglobulin A secretion in the rat cecum. *J. Agric. Food Chem., 59*(10), 5771-5778.

J.Burrell, C., R.Howard, C., & A.Murphy, F. (2016). Mechanisms of Viral Oncogenesis *Fenner and White's Medical Virology* (Fifth ed., pp. 121-134). London, United Kingdom: Academic Press.

Jackson, S. (2007). *Research Methods: A Modular Approach* (3rd ed.). Stamford, CT, USA: Cengage Learning.

Jahromi, M. F., Liang, J. B., Abdullah, N., Goh, Y. M., Ebrahimi, R., & Shokryazdan, P. (2016). Extraction and characterization of oligosaccharides from palm kernel cake as prebiotic. *BioResources, 11*(1), 674-695.

Jahromi, M. F., Shokryazdan, P., Idrus, Z., Ebrahimi, R., Bashokouh, F., & Liang, J. B. (2017). Modulation of Immune Function in Rats Using Oligosaccharides Extracted from Palm Kernel Cake. *Biomed Res Int.*

Jahromi, M. F., Shokryazdan, P., Idrus, Z., Ebrahimi, R., & Liang, J. B. (2017). In Ovo and dietary administration of oligosaccharides extracted from palm kernel cake influence general health of pre-and neonatal broiler chicks. *PloS one, 12*(9), e0184553.

Janeway, C. A., Capra, J. D., Travers, P., & Walport, M. (1999). *Immunobiology: the immune system in health and disease*. NY, USA: Taylor & Francis Group.

Jankowska, A., Laubitz, D., Antushevich, H., Zabielski, R., & Grzesiuk, E. (2008). Competition of *Lactobacillus paracasei* with *Salmonella enterica* for adhesion to Caco-2 cells. *J Biomed Biotechnol, 2008*.

Jenne, C. N., Liao, S., & Singh, B. (2018). Neutrophils: multitasking first responders of immunity and tissue homeostasis: Springer.

Jin, M., Iwamoto, T., Yamada, K., Satsu, H., Totsuka, M., & Shimizu, M. (2011). Effects of chondroitin sulfate and its oligosaccharides on Toll-like receptor-mediated IL-6 secretion by macrophage-like J774. 1 cells. *Biosci., Biotechnol., Biochem., 75*(7), 1283-1289.

Joesten, M. D., Hogg, J. L., & Castellion, M. E. (2007). *The World of Chemistry: Essentials: Essentials* (4th Edition ed.). Belmont, CA, US: Thomson Brooks/Cole.

Jones, G. H., & Ballou, C. E. (1969). Studies on the structure of yeast mannan I. Purification and some properties of an α-mannosidase from an Arthrobacter species. *J Biol Chem., 244*(4), 1043-1051.

Jovanovic-Malinovska, R., Kuzmanova, S., & Winkelhausen, E. (2014). Oligosaccharide profile in fruits and vegetables as sources of prebiotics and functional foods. *Int J Food Prop, 17*(5), 949-965.

Jung, C., Hugot, J.-P., & Barreau, F. (2010). Peyer's patches: the immune sensors of the intestine. *Int J Inflam, 2010*.

Jungmann, J., Rayner, J. C., & Munro, S. (1999). The Saccharomyces cerevisiae protein Mnn10p/Bed1p is a subunit of a Golgi mannosyltransferase complex. *J Biol Chem., 274*(10), 6579-6585.

Kabat, A. M., Pott, J., & Maloy, K. J. (2016). The mucosal immune system and its regulation by autophagy. *Front Immunol, 7*, 240.

Kalidas, N. R., Saminathan, M., Ismail, I. S., Abas, F., Maity, P., Islam, S. S., . . . Shaari, K. (2017). Structural characterization and evaluation of prebiotic activity of oil palm kernel cake mannanoligosaccharides. *Food Chem., 234*, 348-355.

Kandasamy, S., Chattha, K. S., Vlasova, A. N., Rajashekara, G., & Saif, L. J. (2014). Lactobacilli and Bifidobacteria enhance mucosal B cell responses and differentially modulate systemic antibody responses to an oral human rotavirus vaccine in a neonatal gnotobiotic pig disease model. *Gut microbes, 5*(5), 639-651.

Kaplan, H., & Hutkins, R. W. (2000). Fermentation of fructooligosaccharides by lactic acid bacteria and bifidobacteria. *Appl Environ Microbiol, 66*(6), 2682-2684.

Karlsson, J., von Hofsten, J., & Olsson, P.-E. (2001). Generating transparent zebrafish: a refined method to improve detection of gene expression during embryonic development. *Mar Biotechnol, 3*(6), 522-527.

Kaulpiboon, J., Rudeekulthamrong, P., Watanasatitarpa, S., Ito, K., & Pongsawasdi, P. (2015). Synthesis of long-chain isomaltooligosaccharides from tapioca starch and an in vitro investigation of their prebiotic properties. *J Mol Catal B Enzym, 120*, 127-135.

Kaur, G., & Dufour, J. M. (2012). Cell lines: Valuable tools or useless artifacts: Taylor & Francis.

Kengla, C., Kidiyoor, A., & Murphy, S. V. (2017). Bioprinting complex 3D tissue and organs *Kidney Transplantation, Bioengineering and Regeneration* (pp. 957-971). UK: Elsevier.

Khan, C. M. (2014). The dynamic interactions between *Salmonella* and the microbiota, within the challenging niche of the gastrointestinal tract. *Int Sch Res Notices, 2014*.

Khan, F. R., & Alhewairini, S. (2018). Zebrafish (*Danio rerio*) as a model organism. *Curr Trends Cancer Manag*, 3-18.

Kleiveland, C. (2015). Co-culture Caco-2/Immune Cells. In K. Verhoeckx, P. Cotter, I. López-Expósito, C. Kleiveland, T. Lea, A. Mackie, T. Requena, D. Swiatecka, & H. Wichers (Eds.), *The Impact of Food Bioactives on Health: in vitro and ex vivo models* (pp. 197-205). Switzerland: Springer International Publishing.

Kolios, G., Valatas, V., & Ward, S. G. (2004). Nitric oxide in inflammatory bowel disease: a universal messenger in an unsolved puzzle. *Immunology, 113*(4), 427-437.

Kothari, D., Patel, S., & Goyal, A. (2014). Therapeutic spectrum of nondigestible oligosaccharides: overview of current state and prospect. *J Food Sci., 79*(8), R1491-R1498.

Koussounadis, A., Langdon, S. P., Um, I. H., Harrison, D. J., & Smith, V. A. (2015). Relationship between differentially expressed mRNA and mRNA-protein correlations in a xenograft model system. *Scientific reports, 5*(1), 1-9.

Kovacs-Nolan, J., Kanatani, H., Nakamura, A., Ibuki, M., & Mine, Y. (2013). β-1, 4-Mannobiose Stimulates Innate Immune Responses and Induces TLR4-Dependent Activation of Mouse Macrophages but Reduces Severity of Inflammation during Endotoxemia in Mice–3. *J Nutr, 143*(3), 384-391.

Ktsoyan, Z., Ghazaryan, K., Manukyan, G., Martirosyan, A., Mnatsakanyan, A., Arakelova, K., . . . Boyajyan, A. (2013). Inflammatory responses to Salmonella infections are serotype-specific. *Int J Bacteriol, 2013*.

Kurath, G., & Adams, A. (2011). Foreword: Pathogens and immune responses of fish and reptiles: BioMed Central.

Lacy, P., & Stow, J. L. (2011). Cytokine release from innate immune cells: association with diverse membrane trafficking pathways. *Blood, 118*(1), 9-18.

Lardelli, M. (2017). *Using zebrafish in human disease research: some advantages, disadvantages and ethical considerations.* Paper presented at the Proceedings of 2008 ANZCCART Conference, Auckland, New Zealand.

Lea, T. (2015). Caco-2 Cell Line. In K. Verhoeckx, P. Cotter, I. López-Expósito, C. Kleiveland, T. Lea, A. Mackie, T. Requena, D. Swiatecka, & H. Wichers (Eds.), *The Impact of Food Bioactives on Health: in vitro and ex vivo models* (pp. 103-111). Cham: Springer International Publishing.

Lee, S.-H. (2017). Developmental toxicity and anti-inflammatory effect of the soft coral Dendronephthya gigantea collected from Jeju Island in zebrafish model. *Fish Aquatic Sci, 20*(1), 32.

Lepiller, S., Laurens, V., Bouchot, A., Herbomel, P., Solary, E., & Chluba, J. (2007). Imaging of nitric oxide in a living vertebrate using a diaminofluorescein probe. *Free Radic Biol Med, 43*(4), 619-627.

Lesage, G., & Bussey, H. (2006). Cell wall assembly in Saccharomyces cerevisiae. *Microbiol. Mol. Biol. Rev., 70*(2), 317-343.

Levraud, J.-P., Colucci-Guyon, E., Redd, M. J., Lutfalla, G., & Herbomel, P. (2008). In vivo analysis of zebrafish innate immunity *Innate Immunity* (pp. 337-363): Springer.

Liu, M., Luo, F., Ding, C., Albeituni, S., Hu, X., Ma, Y., . . . Jain, D. (2015). Dectin-1 activation by a natural product β-glucan converts immunosuppressive macrophages into an M1-like phenotype. *J. Immunol., 195*(10), 5055-5065.

Livestock farming. (2015). Retrieved from https://www.government.nl/topics/agriculture-and-livestock/contents/livestock-farming

Ljung, T., Lundberg, S., Varsanyi, M., Johansson, C., Schmidt, P. T., Herulf, M., . . . Hellström, P. M. (2006). Rectal nitric oxide as biomarker in the treatment of inflammatory bowel disease: responders versus nonresponders. *World J Gastroenterol, 12*(21), 3386.

Lockyer, S., & Stanner, S. (2019). Prebiotics–an added benefit of some fibre types. *Nutr Bull, 44*(1), 74-91.

López Nadal, A., Ikeda-Ohtsubo, W., Sipkema, D., Peggs, D., McGurk, C., Forlenza, M., . . . Brugman, S. (2020). Feed, microbiota, and gut immunity: using the zebrafish model to understand fish health. *Front Immunol, 11*, 114.

Lowry, V., Farnell, M., Ferro, P., Swaggerty, C., Bahl, A., & Kogut, M. (2005). Purified β-glucan as an abiotic feed additive up-regulates the innate immune response in immature chickens against *Salmonella enterica serovar* Enteritidis. *Int J Food Microbiol, 98*(3), 309-318.

Luiking, Y. C., Engelen, M. P., & Deutz, N. E. (2010). Regulation of nitric oxide production in health and disease. *Curr Opin Clin Nutr Metab Care, 13*(1), 97.

Löfström, C., Hoorfar, J., Schelin, J., Rådström, P., & Malorny, B. (2009). *Salmonella*. In D. Liu (Ed.), *Molecular Detection of Foodborne Pathogens* (pp. 447-449). Boca Raton, FL: CRC Press.

Maccubbin, A., & Black, J. (1986). Passive perchorionic carcinogen bioassay using rainbow trout (*Salmo gairdneri*) embryos. In T. M. Poston & R. Purdy (Eds.), *Aquatic Toxicology and Environmental Fate: Ninth Volume*

(Vol. 9, pp. 277-286). West Conshohocken, PA, USA: ASTM International.

Macpherson, A. J., & Smith, K. (2006). Mesenteric lymph nodes at the center of immune anatomy. *J Exp Med, 203*(3), 497-500.

Mahlapuu, M., Håkansson, J., Ringstad, L., & Björn, C. (2016). Antimicrobial peptides: an emerging category of therapeutic agents. *Front Cell Infect Microbiol, 6*, 194.

Mak, T. W., Jett, B. D., Saunders, M. E., Chaddah, M. R., & Vitetta, E. (2008). Components of the immune system *Primer to The Immune Response* (pp. 38). China: Elsevier Science.

Mantis, N. J., & Corthésy, B. (2007). IgA and Antigen Sampling *Mucosal Immune Defense: Immunoglobulin A* (pp. 203-220): Springer.

Marcus, S. L., Brumell, J. H., Pfeifer, C. G., & Finlay, B. B. (2000). Salmonella pathogenicity islands: big virulence in small packages. *Microbes Infect., 2*(2), 145-156.

Markowiak, P., & Śliżewska, K. (2018). The role of probiotics, prebiotics and synbiotics in animal nutrition. *Gut Pathog, 10*(1), 21.

Maron, D. F., Smith, T. J., & Nachman, K. E. (2013). Restrictions on antimicrobial use in food animal production: an international regulatory and economic survey. *Global Health, 9*(1), 1-11.

Marzel, A., Desai, P. T., Goren, A., Schorr, Y. I., Nissan, I., Porwollik, S., . . . Gal-Mor, O. (2016). Persistent infections by nontyphoidal Salmonella in humans: epidemiology and genetics. *Clin Infect Dis, 62*(7), 879-886.

Masanetz, S., Preißinger, W., Meyer, H., & Pfaffl, M. (2011). Effects of the prebiotics inulin and lactulose on intestinal immunology and hematology of preruminant calves. *animal, 5*(7), 1099-1106.

Mathias, J. R., Dodd, M. E., Walters, K. B., Yoo, S. K., Ranheim, E. A., & Huttenlocher, A. (2009). Characterization of zebrafish larval inflammatory macrophages. *Dev Comp Immunol, 33*(11), 1212-1217.

Mathias, J. R., Saxena, M. T., & Mumm, J. S. (2012). Advances in zebrafish chemical screening technologies. *Future Med Chem, 4*(14), 1811-1822.

Matty, M. A., Oehlers, S. H., & Tobin, D. M. (2016). Live Imaging of Host–Pathogen Interactions in Zebrafish Larvae *Zebrafish* (pp. 207-223): Springer.

McCurley, A. T., & Callard, G. V. (2008). Characterization of housekeeping genes in zebrafish: male-female differences and effects of tissue type, developmental stage and chemical treatment. *BMC Mol Biol, 9*(1), 102.

McKeen, S., Young, W., Mullaney, J., Fraser, K., McNabb, W. C., & Roy, N. C. (2019). Infant complementary feeding of prebiotics for the microbiome and immunity. *Nutrients, 11*(2), 364.

McNeil, B., Archer, D., Giavasis, I., & Harvey, L. (2013). *Microbial Production of Food Ingredients, Enzymes and Nutraceuticals*. UK: Woodhead Publishing Limited.

McWhorter, A. R., & Chousalkar, K. K. (2015). Comparative phenotypic and genotypic virulence of Salmonella strains isolated from Australian layer farms. *Front Microbiol, 6*, 12.

Melancon, E., Canny, S. G. D. L. T., Sichel, S., Kelly, M., Wiles, T., Rawls, J., . . . Guillemin, K. (2017). Best practices for germ-free derivation and gnotobiotic zebrafish husbandry *Methods in cell biology* (Vol. 138, pp. 61-100): Elsevier.

Meyer, T. S. M., Miguel, A., Fernández, D. E. R., & Ortiz, G. M. D. (2015). Biotechnological production of oligosaccharides—applications in the food industry. *Food Prod Ind, 2*, 25-78.

Millet, S., & Maertens, L. (2011). The European ban on antibiotic growth promoters in animal feed: from challenges to opportunities (Vol. 187(2), pp. 143-144): Vet J.

Monastero, R. N., & Pentyala, S. (2017). Cytokines as biomarkers and their respective clinical cutoff levels. *Int J Inflam, 2017*.

Mookiah, S., Sieo, C. C., Ramasamy, K., Abdullah, N., & Ho, Y. W. (2014). Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens. *J Sci Food Agric, 94*(2), 341-348.

Mosser, D. M., & Edwards, J. P. (2008). Exploring the full spectrum of macrophage activation. *Nat. Rev. Immunol., 8*(12), 958.

Multi-country outbreak of Salmonella Enteritidis infections associated with consumption of eggs from Germany. (2014). Retrieved from http://www.efsa.europa.eu/en/supporting/doc/646e.pdf

Murate, L. S., Paião, F. G., de Almeida, A. M., Berchieri Jr, A., & Shimokomaki, M. (2015). Efficacy of prebiotics, probiotics, and synbiotics on laying hens and broilers challenged with *Salmonella* Enteritidis. *J Poult Sci, 52*(1), 52-56.

Murphy, K. M. (2011). *Janeway's Immunobiology* (8 ed.). Third Avenue, NY, USA: Garland Science, Taylor & Francis Group, LLC.

Nabizadeh, A. (2012). The effect of inulin on broiler chicken intestinal microflora, gut morphology, and performance. *J. Anim. Feed Sci, 21*, 725-734.

Ngo, D.-N., Lee, S.-H., Kim, M.-M., & Kim, S.-K. (2009). Production of chitin oligosaccharides with different molecular weights and their antioxidant effect in RAW 264.7 cells. *J Funct Foods, 1*(2), 188-198.

Niewiesk, S. (2014). Maternal antibodies: clinical significance, mechanism of interference with immune responses, and possible vaccination strategies. *Front Immunol, 5*, 446.

Niness, K. R. (1999). Inulin and oligofructose: what are they? *J Nutr, 129*(7), 1402S-1406s.

Nishio, K., Horie, M., Akazawa, Y., Shichiri, M., Iwahashi, H., Hagihara, Y., . . . Niki, E. (2013). Attenuation of lipopolysaccharide (LPS)-induced cytotoxicity by tocopherols and tocotrienols. *Redox Biol, 1*(1), 97-103.

Niu, N., & Wang, L. (2015). In vitro human cell line models to predict clinical response to anticancer drugs. *Pharmacogenomics, 16*(3), 273-285.

Odenwald, M. A., & Turner, J. R. (2017). The intestinal epithelial barrier: a therapeutic target? *Nature reviews Gastroenterology & hepatology, 14*(1), 9.

Ogando, D., Cella, M., Ribeiro, M. L., Weissmann, C., Aisemberg, J., & Franchi, A. (2004). IL-10 inhibits nitric oxide synthesis in murine uterus. *Neuroimmunomodulation, 11*(2), 127-132.

Oilseeds: World markets and trade. (2021). Retrieved from https://apps.fas.usda.gov/psdonline/circulars/oilseeds.pdf

Okubo, Y., Shibata, N., Ichikawa, T., Chaki, S., & Suzuki, S. (1981). Immunochemical study on bakers' yeast mannan prepared by fractional precipitation with cetyltrimethylammonium bromide. *Arch Biochem Biophys, 212*(1), 204-215.

Old, D. (1972). Inhibition of the interaction between fimbrial haemagglutinins and erythrocytes by D-mannose and other carbohydrates. *Microbiol., 71*(1), 149-157.

One of the world's largest palm oil exporter. (2012). Retrieved from http://www.mpoc.org.my/Malaysian_Palm_Oil_Industry.aspx

Otieno, D. O., & Ahring, B. K. (2012). The potential for oligosaccharide production from the hemicellulose fraction of biomasses through pretreatment processes: xylooligosaccharides (XOS), arabinooligosaccharides (AOS), and mannooligosaccharides (MOS). *Carbohydr. Res., 360*, 84-92.

Otte, J., Roland-Holst, D., Pfeiffer, D., Soares-Magalhaes, R., Rushton, J., Graham, J., & Silbergeld, E. (2007). Industrial livestock production and global health risks. *Food and Agriculture Organization of the United Nations, Pro-Poor Livestock Policy Initiative Research Report*.

Paltseva, J., Searle, S., & Malins, C. (2016). Potential for advanced biofuel production from palm residues in Indonesia. *Washington, DC: The International Council on Clean Transportation*.

Panesar, P. S., & Marwaha, S. S. (2013). *Biotechnology in Agriculture and Food Processing: Opportunities and Challenges*. FL, USA: CRC Press.

Parker, H., Albrett, A. M., Kettle, A. J., & Winterbourn, C. C. (2012). Myeloperoxidase associated with neutrophil extracellular traps is active and mediates bacterial killing in the presence of hydrogen peroxide. *J Leukoc Biol, 91*(3), 369-376.

Pasqualetti, V., Altomare, A., Guarino, M. P. L., Locato, V., Cocca, S., Cimini, S., . . . Cicala, M. (2014). Antioxidant activity of inulin and its role in the prevention of human colonic muscle cell impairment induced by lipopolysaccharide mucosal exposure. *PloS one, 9*(5), e98031.

Patel, S., & Goyal, A. (2012). The current trends and future perspectives of prebiotics research: a review. *3 Biotech, 2*(2), 115-125.

Pereira, B. A., Lister, N. L., De-Juan-Pardo, E. M., Risbridger, G. P., & Ellem, S. J. (2017). Three-dimensional bioengineered cancer models *Comprehensive Biomaterials II* (pp. 303-328): Elsevier.

Perumalla, A., Hettiarachchy, N. S., & Ricke, S. C. (2012). Current perspectives on probiotics in poultry preharvest food safety *Direct-Fed Microbials and Prebiotics for Animals* (pp. 89-120): Springer.

Peterson, D. A., McNulty, N. P., Guruge, J. L., & Gordon, J. I. (2007). IgA response to symbiotic bacteria as a mediator of gut homeostasis. *Cell Host Microbe, 2*(5), 328-339.

Pham, L. N., Kanther, M., Semova, I., & Rawls, J. F. (2008). Methods for generating and colonizing gnotobiotic zebrafish. *Nat Protoc, 3*(12), 1862-1875.

Phasing Out Certain Antibiotic Use in Farm Animals. (2013). Retrieved from http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm378100.htm

Phillips, G. O., & Williams, P. A. (2009). *Handbook of Hydrocolloids*: Elsevier Science.

Poultry meat. (2015). from Food And Agriculture Organization Of The United Nations

Statistics Division (FAOSTAT) http://faostat3.fao.org/download/Q/QL/E

Pradhan, A., Khalaf, H., Ochsner, S. A., Sreenivasan, R., Koskinen, J., Karlsson, M., . . . Olsson, P.-E. (2012). Activation of NF-κB protein prevents the transition from juvenile ovary to testis and promotes ovarian development in zebrafish. *J Biol Chem., 287*(45), 37926-37938.

Protopapas, A., Vradelis, S., Karampitsakos, T., Chatzimichail, A., & Paraskakis, E. (2016). Elevated levels of alveolar nitric oxide in patients with inflammatory bowel disease: Eur Respiratory Soc.

Rabsch, W., Andrews, H. L., Kingsley, R. A., Prager, R., Tschäpe, H., Adams, L. G., & Bäumler, A. J. (2002). Salmonella enterica serotype Typhimurium and its host-adapted variants. *Infect Immun, 70*(5), 2249-2255.

Rabsch, W., Fruth, A., Simon, S., Szabo, I., & Malorny, B. (2014). The zoonotic agent *Salmonella*. In A. Sing (Ed.), *Zoonoses - Infections Affecting Humans and Animals: Focus on Public Health Aspects* (pp. 180-182). New York, NY: Springer Netherlands.

Raman, M., Ambalam, P., & Doble, M. (2015). *Probiotics and Bioactive Carbohydrates in Colon Cancer Management*: Springer India.

Ramana, K. V., Fadl, A. A., Tammali, R., Reddy, A. B., Chopra, A. K., & Srivastava, S. K. (2006). Aldose reductase mediates the lipopolysaccharide-induced release of inflammatory mediators in RAW264. 7 murine macrophages. *J Biol Chem., 281*(44), 33019-33029.

Ran, C., Hu, J., Liu, W., Liu, Z., He, S., Dan, B. C. T., . . . Zhou, Z. (2016). Thymol and carvacrol affect hybrid tilapia through the combination of direct stimulation and an intestinal microbiota-mediated effect: insights from a germ-free zebrafish model. *The Journal of nutrition, 146*(5), 1132-1140.

Rauw, W. M. (2012). Immune response from a resource allocation perspective. *Front Genet, 3*, 267.

Raven, P., & Johnson, G. (2002). *Biology* Retrieved from http://www.mhhe.com/biosci/genbio/raven6tour/home.html

Rawls, J. F., Samuel, B. S., & Gordon, J. I. (2004). Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. *Proc Natl Acad Sci U S A, 101*(13), 4596-4601.

Ray, A., & Mitra, A. K. (2017). *Emerging Nanotechnologies for Diagnostics, Drug Delivery and Medical Devices* (A. K. Mitra, K. Cholkar, & A. Mandal Eds.). Amsterdam, Netherlands: Elsevier Science.

Ren, D.-l., Ji, C., Wang, X.-B., Wang, H., & Hu, B. (2017). Endogenous melatonin promotes rhythmic recruitment of neutrophils toward an injury in zebrafish. *Sci Rep, 7*(1), 4696.

Renshaw, S. A., & Trede, N. S. (2012). A model 450 million years in the making: zebrafish and vertebrate immunity. *Dis Model Mech, 5*(1), 38-47.

Rezaei, S., Chen, W., Candyrine, S., Foo, R., Jahromi, M., Farjam, A., . . . Liang, J. (2019). Prebiotic effects of oligosaccharides extracted from palm kernel expeller on different levels of Salmonella typhimurium infection in chicks. *S Afr J Anim Sci, 49*(2), 235-243.

Rezaei, S., Jahromi, M. F., Liang, J. B., Zulkifli, I., Farjam, A. S., Laudadio, V., & Tufarelli, V. (2015). Effect of oligosaccharides extract from palm kernel expeller on growth performance, gut microbiota and immune response in broiler chickens. *Poult Sci, 94*(10), 2414-2420.

Ribeiro, A. M. L., Vogt, L. K., Canal, C. W., Cardoso, M. R. d. I., Labres, R. V., Streck, A. F., & Bessa, M. C. (2007). Effects of prebiotics and probiotics on the colonization and immune response of broiler chickens challenged with *Salmonella* Enteritidis. *Rev Bras Cienc Avic, 9*(3), 193-200.

Ricke, S. C. (2018). Focus: Nutrition and Food Science: Impact of Prebiotics on Poultry Production and Food Safety. *Yale J Biol Med., 91*(2), 151.

Ricke, S. C., & Jarquin, R. (2012). Antimicrobial in animal feed: benefits and limitations. In J. Fink-Gremmels (Ed.), *Animal Feed Contamination: Effects on Livestock and Food Safety* (pp. 412-419). UK: Woodhead Publishing Limited.

Rios, D., Wood, M., Li, J., Chassaing, B., Gewirtz, A., & Williams, I. (2016). Antigen sampling by intestinal M cells is the principal pathway initiating mucosal IgA production to commensal enteric bacteria. *Mucosal Immunol, 9*(4), 907.

Riss, T. L., & Moravec, R. A. (2006). Cell proliferation assays: improved homogeneous methods used to measure the number of cells in culture *Cell biology* (pp. 25-31): Elsevier.

Roberfroid, M. (1993). Dietary fiber, inulin, and oligofructose: a review comparing their physiological effects. *Crit Rev Food Sci Nutr, 33*(2), 103-148.

Roberfroid, M. (2004). *Inulin-Type Fructans: Functional Food Ingredients*: CRC Press.

Roberfroid, M., Gibson, G. R., Hoyles, L., McCartney, A. L., Rastall, R., Rowland, I., . . . Stahl, B. (2010). Prebiotic effects: metabolic and health benefits. *Br J Nutr, 104*(S2), S1-S63.

Rodriguez-Colinas, B., Fernandez-Arrojo, L., Ballesteros, A. O., & Plou, F. J. (2014). Galactooligosaccharides formation during enzymatic hydrolysis of lactose: Towards a prebiotic-enriched milk. *Food Chem, 145*, 388-394.

Rodriguez-Esteban, R., & Jiang, X. (2017). Differential gene expression in disease: a comparison between high-throughput studies and the literature. *BMC medical genomics, 10*(1), 1-10.

Rosales, C. (2018). Neutrophil: a cell with many roles in inflammation or several cell types? *Front Physiol, 9*, 113.

Rosselli, M., Keller, R., & Dubey, R. K. (1998). Role of nitric oxide in the biology, physiology and pathophysiology of reproduction. *Hum Reprod Update, 4*(1), 3-24.

Rushton, J. (2009). *The Economics of Animal Health and Production*. UK: CABI.

Saenphoom, P., Liang, J., Ho, Y., Loh, T., & Rosfarizan, M. (2013). Effects of enzyme treated palm kernel expeller on metabolizable energy, growth performance, villus height and digesta viscosity in broiler chickens. *Asian-Australas J Anim Sci, 26*(4), 537.

Saenphoom, P., Liang, J. H., & Loh, T. R. (2011). Effect of enzyme treatment on chemical composition and production of reducing sugars in palm (Elaeis guineenis) kernel expeller. *Afr J Biotechnol, 10*(68), 15372.

Sahly, H., Keisari, Y., Crouch, E., Sharon, N., & Ofek, I. (2008). Recognition of bacterial surface polysaccharides by lectins of the innate immune system and its contribution to defense against infection: the case of pulmonary pathogens. *Infect Immun, 76*(4), 1322-1332.

Sako, T., Matsumoto, K., & Tanaka, R. (1999). Recent progress on research and applications of non-digestible galacto-oligosaccharides. *Int Dairy J, 9*(1), 69-80.

Salazar, F., Hall, L., Negm, O. H., Awuah, D., Tighe, P. J., Shakib, F., & Ghaemmaghami, A. M. (2016). The mannose receptor negatively modulates the Toll-like receptor 4–aryl hydrocarbon receptor–indoleamine 2, 3-dioxygenase axis in dendritic cells affecting T helper cell polarization. *J Allergy Clin Immunol, 137*(6), 1841-1851. e1842.

Salerno-Goncalves, R., Kayastha, D., Fasano, A., Levine, M. M., & Sztein, M. B. (2019). Crosstalk between leukocytes triggers differential immune responses against Salmonella enterica serovars Typhi and Paratyphi. *PLoS Negl Trop Dis, 13*(8), e0007650.

Salmonella (non-typhoidal). (2018). Retrieved from https://www.who.int/news-room/fact-sheets/detail/salmonella-(non-typhoidal)

Salmonella in livestock production in Great Britain, 2013. (2014). Retrieved 9 March 2015, from Animal Health and Veterinary Laboratories Agency https://www.gov.uk/government/statistics/salmonella-in-livestock-production-in-great-britain-2013

Saman, P., Chaiongkarn, A., Moonmangmee, S., & Artjariyasripong, S. (2017). Prebiotic isomalto-oligosaccharide production from economic crops of Thailand. KKU Res J, 17(5), 794-799.

Santos, M. D., Yasuike, M., Kondo, H., Hirono, I., & Aoki, T. (2008). Teleostean IL11b exhibits complementing function to IL11a and expansive involvement in antibacterial and antiviral responses. *Mol Immunol, 45*(12), 3494-3501.

Santos, R. L., Raffatellu, M., Bevins, C. L., Adams, L. G., Tükel, Ç., Tsolis, R. M., & Bäumler, A. J. (2009). Life in the inflamed intestine, Salmonella style. *Trends Microbiol, 17*(11), 498-506.

Santos, R. L., Tsolis, R. M., Bäumler, A. J., Smith, R., & Adams, L. G. (2001). Salmonella enterica Serovar typhimurium induces cell death in bovine monocyte-derived macrophages by early sipb-dependent and delayedsipb-independent mechanisms. *Infect Immun, 69*(4), 2293-2301.

Santos, R. L., Zhang, S., Tsolis, R. M., Kingsley, R. A., Adams, L. G., & Bäumler, A. J. (2001). Animal models of Salmonella infections: enteritis versus typhoid fever. *Microbes and Infection, 3*(14-15), 1335-1344.

Saraceni, P. R., Romero, A., Figueras, A., & Novoa, B. (2016). Establishment of infection models in zebrafish larvae (Danio rerio) to study the pathogenesis of Aeromonas hydrophila. *Front Microbiol, 7*, 1219.

Schnyder, J., & Baggiolini, M. (1978). Role of phagocytosis in the activation of macrophages. *J. Exp. Med., 148*(6), 1449-1457.

Schultz, B. M., Salazar, G. A., Paduro, C. A., Pardo-Roa, C., Pizarro, D. P., Salazar-Echegarai, F. J., . . . Álvarez-Lobos, M. M. (2018). Persistent Salmonella enterica serovar typhimurium infection increases the susceptibility of mice to develop intestinal inflammation. *Front Immunol, 9*, 1166.

Schürmann, N., Forrer, P., Casse, O., Li, J., Felmy, B., Burgener, A.-V., . . . Hess, C. (2017). Myeloperoxidase targets oxidative host attacks to Salmonella and prevents collateral tissue damage. *Nat Microbiol, 2*(4), 1-9.

Schwan, W. R., Huang, X.-Z., Hu, L., & Kopecko, D. J. (2000). Differential bacterial survival, replication, and apoptosis-inducing ability of Salmonella serovars within human and murine macrophages. *Infect Immun, 68*(3), 1005-1013.

Schwartz, D. A. (2011). Infectious and Parasitic Diseases. In R. Rubin, D. S. Strayer, & E. Rubin (Eds.), *Rubin's Pathology: Clinicopathologic Foundations of Medicine* (6 ed., pp. 359-360). Baltimore, MD: Wolters Kluwer Health/Lippincott Williams & Wilkins.

Searle, L. E., Best, A., Nunez, A., Salguero, F. J., Johnson, L., Weyer, U., . . . Jones, G. (2009). A mixture containing galactooligosaccharide, produced by the enzymic activity of Bifidobacterium bifidum, reduces Salmonella enterica serovar Typhimurium infection in mice. *J Med Microbiol, 58*(1), 37-48.

Searle, L. E., Cooley, W. A., Jones, G., Nunez, A., Crudgington, B., Weyer, U., . . . Woodward, M. J. (2010). Purified galactooligosaccharide, derived from a mixture produced by the enzymic activity of Bifidobacterium bifidum, reduces Salmonella enterica serovar Typhimurium adhesion and invasion in vitro and in vivo. *J Med Microbiol, 59*(12), 1428-1439.

Sebastiani, G., Blais, V., Sancho, V., Vogel, S. N., Stevenson, M. M., Gros, P., . . . Malo, D. (2002). Host immune response to Salmonella enterica serovar Typhimurium infection in mice derived from wild strains. *Infect Immun, 70*(4), 1997-2009.

Selected Agricultural Indicators, Malaysia, 2019. (2019). Retrieved from https://www.dosm.gov.my/v1/index.php?r=column/cthemeByCat&cat=72&bul_id=SEUxMEE3VFdBcDJhdUhPZVUxa2pKdz09&menu_id=Z0VTZGU1UHBUT1VJMFlpaXRRR0xpdz09

Sender, R., Fuchs, S., & Milo, R. (2016). Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol., 14*(8), e1002533.

Shalaei, M., Hosseini, S. M., & Zergani, E. (2014). *Effect of different supplements on eggshell quality, some characteristics of gastrointestinal tract and performance of laying hens.* Paper presented at the Veterinary Research Forum.

Shi, N., Li, N., Duan, X., & Niu, H. (2017). Interaction between the gut microbiome and mucosal immune system. *Mil Med Res, 4*(1), 1-7.

Shoaf-Sweeney, K. D., & Hutkins, R. W. (2008). Adherence, Anti-Adherence, and Oligosaccharides: Preventing Pathogens from Sticking to the Host. *Adv. Food Nutr. Res., 55*, 101-161.

Shukla, P., & Pletschke, B. I. (2013). *Advances in Enzyme Biotechnology*: Springer India.

Sierra, C., Bernal, M.-J., Blasco, J., Martínez, R., Dalmau, J., Ortuño, I., . . . Vidal, M.-L. (2014). Prebiotic effect during the first year of life in healthy infants fed formula containing GOS as the only prebiotic: a multicentre, randomised, double-blind and placebo-controlled trial. *Eur J Nutr, 54*(1), 89-99.

Simon, A. K., Hollander, G. A., & McMichael, A. (2015). Evolution of the immune system in humans from infancy to old age. *Proceedings of the Royal Society B: Biological Sciences, 282*(1821), 20143085.

Siro, I., Kapolna, E., Kapolna, B., & Lugasi, A. (2008). Functional food. Product development, marketing and consumer acceptance—A review. *Appetite, 51*(3), 456-467.

Smith Jr, D. L., Nagy, T. R., Wilson, L. S., Dong, S., Barnes, S., & Allison, D. B. (2010). The effect of mannan oligosaccharide supplementation on body weight gain and fat accrual in C57Bl/6J mice. *Obesity, 18*(5), 995-999.

Sohail, M., Ijaz, A., Younus, M., Shabbir, M., Kamran, Z., Ahmad, S., . . . Shahzad, A. (2013). Effect of supplementation of mannan oligosaccharide and probiotic on growth performance, relative weights of viscera, and population of selected intestinal bacteria in cyclic heat-stressed broilers. *J Appl Poult Res, 22*(3), 485-491.

Solano, C., Sesma, B., Alvarez, M., Urdaneta, E., Garcia-Ros, D., Calvo, A., & Gamazo, C. (2001). Virulent strains of *Salmonella* Enteritidis disrupt the epithelial barrier of Caco-2 and HEp-2 cells. *Arch Microbiol, 175*(1), 46-51.

Soleimani, N., Hoseinifar, S. H., Merrifield, D. L., Barati, M., & Abadi, Z. H. (2012). Dietary supplementation of fructooligosaccharide (FOS) improves the innate immune response, stress resistance, digestive enzyme activities and growth performance of Caspian roach (Rutilus rutilus) fry. *Fish Shellfish Immunol, 32*(2), 316-321.

Soni, H., & Kango, N. (2013). *Microbial Mannanases: Properties and Applications.* . Haryana, India: Springer.

Spacova, I., Dodiya, H. B., Happel, A.-U., Strain, C., Vandenheuvel, D., Wang, X., & Reid, G. (2020). Future of probiotics and prebiotics and the implications for early career researchers. *Frontiers in microbiology, 11*.

Stanaway, J. D., Parisi, A., Sarkar, K., Blacker, B. F., Reiner, R. C., Hay, S. I., . . . Mokdad, A. H. (2019). The global burden of non-typhoidal salmonella invasive disease: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Infect Dis, 19*(12), 1312-1324.

Stanton, Emms, & Sia. (2011). Malaysia′ s markets for functional foods, nutraceuticals and organic foods: An introduction for Canadian producers and exporters. March 2011.

Stecher, B., Maier, L., & Hardt, W.-D. (2013). 'Blooming'in the gut: how dysbiosis might contribute to pathogen evolution. *Nat. Rev. Microbiol., 11*(4), 277.

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M., & Haan, C. d. (2006). *Livestock's long shadow: environmental issues and options*: Food and Agriculture Organization of the United Nations (FAO).

Sullivan, C., & Kim, C. H. (2008). Zebrafish as a model for infectious disease and immune function. *Fish & shellfish immunology, 25*(4), 341-350.

Summers, S. A., Steinmetz, O. M., Gan, P. Y., Ooi, J. D., Odobasic, D., Kitching, A. R., & Holdsworth, S. R. (2011). Toll-like receptor 2 induces Th17 myeloperoxidase autoimmunity while Toll-like receptor 9 drives Th1 autoimmunity in murine vasculitis. *Arthritis Rheum, 63*(4), 1124-1135.

Suzuki, S. (1994). Pathogenicity of *Salmonella* Enteritidis in poultry. *Int J Food Microbiol, 21*(1-2), 89-105.

Tan, F., Limbu, S. M., Qian, Y., Qiao, F., Du, Z.-Y., & Zhang, M. (2019). The responses of germ-free zebrafish (Danio rerio) to varying bacterial concentrations, colonization time points, and exposure duration. *Frontiers in microbiology, 10*, 2156.

Tan, Y.-A. (2006). By-products of palm oil extraction and refining. *Oléagineux, Corps gras, Lipides, 13*(1), 9-11.

Tanaka, T., Narazaki, M., & Kishimoto, T. (2014). IL-6 in inflammation, immunity, and disease. *Cold Spring Harbor perspectives in biology, 6*(10), a016295.

Taub, D. D., Anver, M., Oppenheim, J. J., Longo, D. L., & Murphy, W. J. (1996). T lymphocyte recruitment by interleukin-8 (IL-8). IL-8-induced degranulation of neutrophils releases potent chemoattractants for human T lymphocytes both in vitro and in vivo. *J Clin Invest, 97*(8), 1931-1941.

Ten Bruggencate, S. J., Bovee-Oudenhoven, I. M., Lettink-Wissink, M. L., & Van der Meer, R. (2003). Dietary fructo-oligosaccharides dose-dependently increase translocation of salmonella in rats. *J Nutr, 133*(7), 2313-2318.

Ten Bruggencate, S. J., Bovee-Oudenhoven, I. M., Lettink-Wissink, M. L., & Van der Meer, R. (2005). Dietary fructooligosaccharides increase intestinal permeability in rats. *J Nutr, 135*(4), 837-842.

Thai, T. H., Hirai, T., Lan, N. T., & Yamaguchi, R. (2012). Antibiotic resistance profiles of Salmonella serovars isolated from retail pork and chicken meat in North Vietnam. *Int. J. Food Microbiol., 156*(2), 147-151.

Thammana, M. (2016). A review on high performance liquid chromatography (HPLC). *RRJPA, 5*(2), 22-28.

Thiennimitr, P., Winter, S. E., Winter, M. G., Xavier, M. N., Tolstikov, V., Huseby, D. L., . . . Bäumler, A. J. (2011). Intestinal inflammation allows Salmonella to use ethanolamine to compete with the microbiota. *Proc Natl Acad Sci U S A, 108*(42), 17480-17485.

Thornton, P. K. (2010). Livestock production: recent trends, future prospects. *Philos Trans R Soc Lond B Biol Sci, 365*(1554), 2853-2867.

Thung, T., Mahyudin, N., Basri, D. F., Wan Mohamed Radzi, C., Nakaguchi, Y., Nishibuchi, M., & Radu, S. (2016). Prevalence and antibiotic resistance of *Salmonella* Enteritidis and *Salmonella* Typhimurium in raw chicken meat at retail markets in Malaysia. *Poult. Sci., 95*(8), 1888-1893.

Tisch, D. (2005). *Animal Feeds, Feeding and Nutrition, and Ration Evaluation CD-ROM*: Cengage Learning.

Titheradge, M. A. (1999). Nitric oxide in septic shock. *Biochim Biophys Acta Bioenerg, 1411*(2-3), 437-455.

Torres, D. P., Gonçalves, M. d. P. F., Teixeira, J. A., & Rodrigues, L. R. (2010). Galacto-Oligosaccharides: Production, Properties, Applications, and Significance as Prebiotics. *Compr Rev Food Sci Food Saf, 9*(5), 438-454.

Tovey, M. G., & Lallemand, C. (2011). Immunogenicity and other problems associated with the use of biopharmaceuticals. *Ther Adv Drug Saf, 2*(3), 113-128.

Trisakti, B., Lubis, J., & Husaini, T. (2017). *Effect of Turning Frequency on Composting of Empty Fruit Bunches Mixed with Activated Liquid Organic Fertilizer.* Paper presented at the IOP Conference Series: Materials Science and Engineering.

Troxell, B., Petri, N., Daron, C., Pereira, R., Mendoza, M., Hassan, H. M., & Koci, M. D. (2015). Poultry body temperature contributes to invasion control through reduced expression of Salmonella pathogenicity island 1 genes in *Salmonella* enterica serovars Typhimurium and Enteritidis. *Appl Environ Microbiol, 81*(23), 8192-8201.

Tükel, Ç., Raffatellu, M., Chessa, D., Wilson, R. P., Akçelik, M., & Bäumler, A. J. (2006). Neutrophil influx during non-typhoidal salmonellosis: who is in the driver's seat? *FEMS Immunol Med Microbiol, 46*(3), 320-329.

Tyrkalska, S. D., Candel, S., Angosto, D., Gómez-Abellán, V., Martín-Sánchez, F., García-Moreno, D., . . . Pelegrín, P. (2016). Neutrophils mediate Salmonella Typhimurium clearance through the GBP4 inflammasome-dependent production of prostaglandins. *Nat Commun, 7*(1), 1-17.

Uzal, F., Plattner, B., & Hostetter, J. (2007). Alimentary System. In J. Caswell, K. Williams, & M. Maxie (Eds.), *Jubb, Kennedy and Palmer's Pathology of Domestic Animals* (6 ed., Vol. 2, pp. 167-168).

Valente, K. P., Khetani, S., Kolahchi, A. R., Sanati-Nezhad, A., Suleman, A., & Akbari, M. (2017). Microfluidic technologies for anticancer drug studies. *Drug discovery today, 22*(11), 1654-1670.

Valle, E., & Guiney, D. G. (2005). Characterization of Salmonella-induced cell death in human macrophage-like THP-1 cells. *Infect Immun, 73*(5), 2835-2840.

Valpotić, H., Samardžija, M., Terzić, S., Vince, S., Šperanda, M., Lacković, G., . . . Kočila, P. (2016). Effect of mannan oligosaccharide supplementation on blood and intestinal immune cells, bacteria numbers and performance in weaned pigs. *Acta Veterinaria Brno, 85*(3), 267-276.

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., . . . Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proc Natl Acad Sci U S A, 112*(18), 5649-5654.

Van den Ende, W., Peshev, D., & De Gara, L. (2011). Disease prevention by natural antioxidants and prebiotics acting as ROS scavengers in the gastrointestinal tract. *Trends Food Sci Technol, 22*(12), 689-697.

Van Loo, J., Coussement, P., De Leenheer, L., Hoebregs, H., & Smits, G. (1995). On the presence of inulin and oligofructose as natural ingredients in the western diet. *Crit Rev Food Sci Nutr, 35*(6), 525-552.

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., & Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol, 3*(7), research0034. 0031.

Varas, M., Fariña, A., Díaz-Pascual, F., Ortíz-Severín, J., Marcoleta, A. E., Allende, M. L., . . . Chávez, F. P. (2017). Live-cell imaging of Salmonella Typhimurium interaction with zebrafish larvae after injection and immersion delivery methods. *J Microbiol Methods, 135*, 20-25.

Vargas, F., Moreno, J. M., Wangensteen, R., Rodríguez-Gómez, I., & García-Estañ, J. (2007). The endocrine system in chronic nitric oxide deficiency. *Eur J Endocrinol, 156*(1), 1-12.

Veereman, G. (2007). Pediatric applications of inulin and oligofructose. *J Nutr, 137*(11), 2585S-2589S.

Vergauwen, H. (2015). The IPEC-J2 Cell Line. In K. Verhoeckx (Ed.), *The Impact of Food Bio-Actives on Gut Health In Vitro and Ex Vivo Models* (pp. 128). Switzerland: Springer.

Verma, A. (2014). Animal tissue culture: Principles and applications *Animal Biotechnology* (pp. 211-231): Elsevier.

Vispo, C. K., W.H. (2012). Interactions of Avian Gut Microbes and Their Host: An Elusive Symbiosis. In R. Mackie & B. White (Eds.), *Gastrointestinal Microbiology: Volume 1 Gastrointestinal Ecosystems and Fermentations* (Vol. 1, pp. 117-120): Springer US.

Wang, C.-N., Duan, G.-L., Liu, Y.-J., Yu, Q., Tang, X.-L., Zhao, W., . . . Ni, X. (2015). Overproduction of nitric oxide by endothelial cells and macrophages contributes to mitochondrial oxidative stress in adrenocortical cells and adrenal insufficiency during endotoxemia. *Free Radic Biol Med, 83*, 31-40.

Wang, H., Ji, D., Shao, J., & Zhang, S. (2012). Maternal transfer and protective role of antibodies in zebrafish Danio rerio. *Mol Immunol, 51*(3), 332-336.

Wei, T., Chen, C., Hou, J., Xin, W., & Mori, A. (2000). Nitric oxide induces oxidative stress and apoptosis in neuronal cells. *Biochim Biophys Acta Mol Cell Res, 1498*(1), 72-79.

Weitekamp, C. A., Kvasnicka, A., Keely, S. P., Brinkman, N. E., Howey, X. M., Gaballah, S., . . . Wheaton, E. (2021). Monoassociation with bacterial isolates reveals the role of colonization, community complexity and abundance on locomotor behavior in larval zebrafish. *Anim Microbiome, 3*(1), 1-13.

White, L., Newman, M., Cromwell, G., & Lindemann, M. (2002). Brewers dried yeast as a source of mannan oligosaccharides for weanling pigs. *J Anim Sci, 80*(10), 2619-2628.

Wilkowska, A., Nowak, A., Antczak-Chrobot, A., Motyl, I., Czyżowska, A., & Paliwoda, A. (2019). Structurally different pectic oligosaccharides produced from apple pomace and their biological activity in vitro. *Foods, 8*(9), 365.

Winger, M., Christen, M., & van Gunsteren, W. F. (2009). On the conformational properties of amylose and cellulose oligomers in solution. *Int J Carbohydr Chem, 2009*.

Wolf, A., Aggio, J., Campbell, C., Wright, F., Marquez, G., Traver, D., & Stachura, D. L. (2017). Zebrafish caudal haematopoietic embryonic stromal tissue (CHEST) cells support haematopoiesis. *Sci Rep, 7*, 44644.

Wu, C.-C., Chen, J.-S., Wu, W.-M., Liao, T.-N., Chu, P., Lin, S.-H., . . . Lin, Y.-F. (2005). Myeloperoxidase serves as a marker of oxidative stress during single haemodialysis session using two different biocompatible dialysis membranes. *Nephrol Dial Transplant, 20*(6), 1134-1139.

Wu, C., Xue, Y., Wang, P., Lin, L., Liu, Q., Li, N., . . . Cao, X. (2014). IFN-γ primes macrophage activation by increasing phosphatase and tensin homolog via downregulation of miR-3473b. *J Immunol, 193*(6), 3036-3044.

Wu, R.-Q., Zhang, D.-F., Tu, E., Chen, Q.-M., & Chen, W. (2014). The mucosal immune system in the oral cavity—an orchestra of T cell diversity. *Int J Oral Sci, 6*(3), 125.

Wu, R. Y., Määttänen, P., Napper, S., Scruten, E., Li, B., Koike, Y., . . . Botts, S. R. (2017). Non-digestible oligosaccharides directly regulate host kinome to modulate host inflammatory responses without alterations in the gut microbiota. *Microbiome, 5*(1), 135.

Xu, C., & Zon, L. I. (2010). The zebrafish as a model for human disease *Fish Physiol* (Vol. 29, pp. 345-365): Elsevier.

Xu, Z., Hu, C., Xia, M., Zhan, X., & Wang, M. (2003). Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poult Sci, 82*(6), 1030-1036.

Yang, B., Qu, D., Zhang, X., Shen, J., Cui, S., Shi, Y., . . . Meng, J. (2010). Prevalence and characterization of Salmonella serovars in retail meats of marketplace in Shaanxi, China. *Int. J. Food Microbiol., 141*(1-2), 63-72.

Yang, Y., & Ma, H. (2009). Western blotting and ELISA techniques. *Researcher, 1*(2), 67-86.

Yoo, S. K., & Huttenlocher, A. (2011). Spatiotemporal photolabeling of neutrophil trafficking during inflammation in live zebrafish. *J Leukoc Biol, 89*(5), 661-667.

Zahari, M. W., & Alimon, A. (2004). Use of palm kernel cake and oil palm by-products in compound feed. *Palm Oil Dev, 40*, 5-8.

Zhang, J.-M., & An, J. (2007). Cytokines, inflammation and pain. *Int Anesthesiol Clin, 45*(2), 27.

Zhang, T., Pan, Z., Qian, C., & Chen, X. (2009). Isolation and purification of D-mannose from palm kernel. *Carbohydr Res, 344*(13), 1687-1689.

Zhang, Z., Guan, N., Li, T., Mais, D. E., & Wang, M. (2012). Quality control of cell-based high-throughput drug screening. *Acta Pharmaceutica Sinica B, 2*(5), 429-438.

Zou, Y., Fu, X., Liu, N., Duan, D., Wang, X., Xu, J., & Gao, X. (2019). The synergistic anti-inflammatory activities of agaro-oligosaccharides with different degrees of polymerization. *J Appl Phycol*, 1-12.

BIODATA OF STUDENT

Foo Rui Qing (GS42192) is currently a postgraduate student pursuing her Doctor of Philosophy from the Institute of Tropical Agriculture and Food Security (ITAFoS), UPM. Prior to pursuing her PhD, Foo Rui Qing has obtained her M. Sc. Pharmaceutical Chemistry and B. Sc. (Hons) Biotechnology from UCSI University.

In terms of working experiences, Foo Rui Qing has worked as an internship student in Forest Research Institute Malaysia (FRIM) (2007) and Melaka Biotechnology Corporation (2009). She subsequently worked as a Production Executive at National Semiconductor in 2011 before becoming a research assistant for ITAFoS, UPM in 2015.

Foo Rui Qing has technical skills in microbiology, animal and plant cell culture, natural product analysis and antioxidant analysis.

In her free time, the she enjoys reading, writing and drawing. Foo Rui Qing believes in an interdisciplinary approach to knowledge accruement and enjoys listening to talks and lectures from many different fields in order to apply and integrate the knowledge gained into problem solving and research.

LIST OF PUBLICATIONS

Foo, R. Q., Jahromi, M. F., Chen, W. L., Ahmad, S., Lai, K. S., Idrus, Z., & Liang, J. B. (2020). Oligosaccharides from palm kernel cake enhances adherence inhibition and intracellular clearance of *Salmonella enterica* serovar Enteritidis *in vitro*. *Microorganisms, 8*(2), 255.

Foo, R. Q., Ahmad, S., Lai, K. S., Idrus, Z., Yusoff, K., & Liang, J. B. (2020). Palm kernel cake oligosaccharides acute toxicity and effects on nitric oxide levels using a zebrafish larvae model. *Front Physiol, 11.*