UNIVERSITY OF NEW ENGLAND

NOVEL PROBIOTICS FOR BROILER CHICKENS

By

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DECLARATION

I certify that the substance of this thesis has not been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that, to the best of my knowledge, any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

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TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAHL	Australian Animal Health Laboratory
ABC	ATP-binding cassette
AggH	Aggregation helicase
ARDRA	Amplified ribosomal DNA restriction analysis
ANOVA	Analysis of variance
BHI	Brain heart infusion
Bp	Basepair (s)
BWG	Body weight gain
CE	Competitive exclusion
CFU	Colony forming units
CnBP	Collagen-binding protein
C. perfringens	*Clostridium perfringens*
d	day
DFM	Direct-fed microbial
DNA	Deoxyribonucleic acid
dNTP	Deoxynucloside triphosphate
E. coli	*Escherichia Coli*
EDTA	Ethylendiamin-N,N,N',N'-tetra acid
EU	European Union
FCR	Feed conversion ratio
FI	Feed intake
FOS	Fructooligosaccharide products
g	Gram
GALT	Gut-associated lymphoid tissue
GC	Gas chromatography
GIT	Gastrointestinal tract
h	Hour
IFA	In- feed antibiotic

LIST OF ABBREVIATIONS

LAB	Lactic acid bacteria
LB	Luria Bertani
LSD	Least Significant difference
LTA	Lipoteichoic acid
ME	Metabolizable energy
mg	Milligram
MHC	Major histocompatability complex class
min	Minutes
MOS	Mannanoligosaccarides
MQ-H_2O	Milli-Q filtered deionised water
MRS	Man, Rogosa, and Sharp
NARMS	National Antimicrobial Research Monitoring System
NE	Necrotic enteritis
NSP	Non-starch polysaccharides
O.D.	Optic density
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
Ratio	Villus height : crypt depth ratio
TBE	Tris-borate-EDTA
TE	Tris-EDTA
TGGE	Temperature gradient gel electrophoresis
SARDI	South Australia Research and Development Institute
SDS	Sodium dodecyl sulphate
SE	Standard error

LIST OF ABBREVIATIONS

VFAs	Valatile fatty acid
VRE	Vancomycin-resistant enterococci
UK	United Kingdom
UNE	University of New England
USA	United State of America
µg	Microgram
µL	Microlitre
v/v	Volume for volume
WHO	World Health Organization
ZnB	Zinc bacitracin

LIST OF PUBLICATIONS

Olnood, C. G., Mikkelsen, L. L., Choct, M. and Iji, P. A. (2007) Antagonistic activity of novel probiotics and their effect on growth performance of broiler chickens. *Australian Poultry Science Symposium*, Australia, **19**: 153-156.

Olnood, C. G., Mikkelsen, L. L., Choct, M. and Iji, P. A. (2007) Effects of novel probiotics feed additives on pH, volatile fatty acid content and bacteria in the broiler gut. *Recent Advances in Animal Nutrition*, Australia, **16**: 171.

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Olnood, C. G., Choct, M., Iji, P. A. and Mikkelsen, L. L. (2009) Effects of novel probiotic *Lactobacillus johnsonii* on growth performance, gut morphology and intestinal microflora of broiler chickens challenge with *Salmonella sofia*. *Australian Poultry Science Symposium*, 20: (in press).

SUMMARY

The purpose of this thesis was to select beneficial bacteria from the chicken intestinal tract and to screen them for their potential probiotic characteristics in order to use them against pathogenic bacteria, such as *C. perfringens* and *Salmonella*. Five experiments (Chapters 3-7) were conducted and out of four potential probiotic candidates, *L. johnsonii* was eventually selected as a model organism and its effects on bird performance, gut microflora, gut morphology, and antibiotic effect were examined. Throughout this thesis, Cobb, male broilers were used. Each experimental chapter has been presented as a stand-alone paper, thus, this summary will only give an overview of the key findings of the thesis.

Chapter 1 briefly describes the background information and justified the importance of research in the topic of interest, leading to the major hypothesis and objectives for conducting the five experiment contained in the thesis. Chapter 2, the literature review, covers the use of probiotics in poultry production, focusing on their modes of action and properties, and their potential as alternatives to in-feed antibiotics.

In Chapter 3, four probiotic isolates were selected from 235 lactobacillus isolates of poultry origin using an antagonistic test against *C. perfringens, E. coli* and *S. sofia*. Quantitative and qualitative measurements revealed that these four candidates, *L. johnsonii, L. crispatus*, *L. salivarius* and one unidentified *L.* sp., were antagonistic towards *C. perfringens, E. coli* and *S. sofia in vitro* and were able to survive in feed for 7 days, in water and litter for more than 24 hours under practical production conditions.

Chapter 4 presents data the efficacy of delivering *L. johnsonii, L. crispatus*, *L. salivarius* and an unidentified *Lactobacillus* sp. via feed in manipulating gut microflora environment and production performance was investigated. Results showed that none of the four candidates improved bird performance but they increased the small intestinal weight and tended to reduce the number of enterobacteria in the ileum. Among the four candidates, *L. johnsonii* was the best in its effects on gut development and gut microflora, thus it alone was to be used in subsequent studies.

Chapter 5 presents results related to the efficacy in delivering *L. johnsonii*, via four delivery routes, *i. e*., feed, water, litter and oral gavage in bird performance, gut development and gut microflora. Consistent with previous findings, *L. johnsonii* did not improve bird performance regardless of the route of administration. It, however, reduced the number of enterobacteria in the caeca on d 21, and strongly tended to reduced it in the ileum and caeca on d 7 and in the ileum on d 21 compared with the control. The probiotic also tended to increase the number of lactic acid bacteria and lactobacilli in the ileum and caeca on d 7, but this trend was not evident on d 21. The trend appeared most pronounced when the probiotic was delivered orally or via litter. Delivery of the probiotic through feed, water and litter increased ($p<0.01$) the pancreas weight on d 21, but the probiotic did not affect other morphometric parameters of the gut.

Chapters 6 and 7 present the findings on the effects of *L. johnsonii* on gut microflora, bird performance and intestinal development using two challenge trials, *i.e.*, *Salmonella sofia* and *Clostridium perfringens,* respectively. In Chapter 6, *L. johnsonii* reduced the number of *S. sofia* and *C. perfringens* in the gut environment, and improved the birds' resistance to *S. sofia*, whereas in Chapter 7 birds orally inoculated with *L. johnsonii* had an enhanced absorptive capacity of the small intestine, numerically reduced the *C. perfringens* counts and NE lesion scores in the gut, and improved body weight gain of broilers under NE challenge. But the probiotic failed to completely protect birds from necrotic enteritis.

Finally, Chapter 8 discusses the major implications and significance of the thesis in a concise and coordinated manner.

It may be concluded that there is a significant scope to use probiotics to manipulate gut microflora, and with time, it is possible to fine tune it to produce specific health and production outcomes in poultry. However, the effect of four selected lactobacilli of poultry origin on bird performance was not profound albeit positive, particularly under challenge situations. Based on the findings in this thesis, there is still some way to go in terms of finding truly efficacious probiotics that have growth promoting properties and the ability to protect birds from significant pathogen challenge such as necrotic enteritis.