Functional Interactions of Mannooligosaccharides with Dietary Threonine on Chicken Gastrointestinal Tract

By

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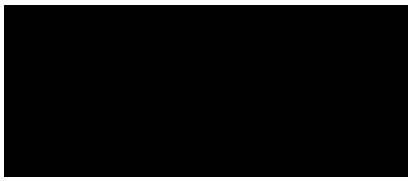
DECLARATION

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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LIST OF ABBREVIATIONS

A	Absorbances
AA	Amino acid(s)
AB	Alcian blue
AID	Apparent ileal digestibility
AME_n	nitrogen-corrected Apparent metabolisable energy
ANOVA	Analysis of variance
AUD	Australian Dollar
bp	Base pair
BSH	Bile salt hydrolase
BWG	Body weight gain
C	Carbon
CFU	Colony forming unit
CP	Crude protein
C_s	Similarity (Sorenson's) coefficients
CSIRO	Commonwealth Scientific and Industrial Research Organisation
C_T	Threshold cycle
d	Day(s)
Da	Dalton
DGGE	Denaturing gradients gel electrophoresis
DM	Dry matter
DNA	Deoxyribonucleic acid
cDNA	complementary Deoxyribonucleic acid
rDNA	ribosomal Deoxyribonucleic acid
EF	Endogenous flow
EHC	Enzymatic hydrolysed casein
FCR	Feed conversion ratio
FI	Feed intake
FISH	Fluorescent *in situ* hybridisation
GIT	Gastrointestinal tract
GLM	General Linear Model
h	Hour(s)
HID	High iron diamine
IOD	Integrated optical density
IU	International Units

ME	Metabolisable energy
min	Minutes
MOS	Mannooligosaccharides
mRNA	messenger Ribonucleic acid
MUC	Mucin gene
NC	Negative control
NRC	National Research Council
PAS	Periodic acid-Schiff's
PCR	Polymerase chain reaction
PER	Protein efficiency ratio
ppm	Parts per million
RDP	Ribosomal Database Project
rRNA	ribosomal Ribonucleic acid
RT-PCR	Reverse transcription-polymerase chain reaction
s	Second(s)
SCFA	Short-chain fatty acid(s)
SD	Standard deviation
SEM	Standard error mean (Pooled)
SID	Standardised ileal digestibility
spp.	Species
SSCP	Single-strand conformation polymorphism
subsp.	Sub-species
TER	Threonine efficiency ratio
TGGE	Temperature gradients gel electrophoresis
Thr	Threonine
TiO_2	Titanium dioxide
T-RFLP	Terminal-restriction fragment length polymorphism
T-RFs	Terminal-restricted fragments
TTGE	Temporal thermal gradient gel electrophoresis
UNE	University of New England
VFA	Volatile fatty acid(s)
VWD	von Willebrand factor type D domain
ZnB	Zinc bacitracin

LIST OF PUBLICATIONS

Chee, S.H. Iji, P.A. Choct, M. and Mikkelsen, L.L. (2007). Dietary threonine deficiency specifically impairs intestinal mucin synthesis at translational level in broiler chicken. In: *Proceedings of Recent Advances in Animal Nutrition*. **16**:274.

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SUMMARY

This thesis presents a literature review and reports on four experiments conducted to investigate the interactive effects of MOS (Bio-MOS®, Alltech Biotechnology) with dietary threonine on the growth performance of broiler chickens. To elucidate the mechanisms underlying these effects, a series of other physiological responses in relation to the intestinal mucin dynamics, gut development, gut microflora, nutrient utilisation, and feed passage rate were examined.

Chapter 1 briefly outlines the background information leading to the formation of the major hypotheses and objectives of the present studies. Chapter 2 is a literature review on gut microflora and development, mucin dynamics and influence of MOS and dietary threonine on intestinal mucin turnover.

Chapter 3 underlines the importance of optimising intestinal mucin dynamics and gut development for the maximisation of growth. Positive interactions between MOS and threonine supplements on body weight gain (BWG) were apparent in all phases of growth with the highest BWG noticed in birds given the excess-threonine plus MOS diet. Graded levels of dietary threonine induced a quadratic growth response, whereas the growth-promoting effects of MOS were both age-dependent and threonine sensitive, especially at the later stage of growth. Thus, feeding of MOS with threonine supplemented to meet the extra amount demanded by MOS resulted in an additional BWG and better feed conversion ratio (FCR) and was economically justified based on the improved net profit return. Dietary threonine greatly influenced mucin synthesis at the translational stage. Conversely, MOS modulated the transcriptional stage of intestinal mucin synthesis. Dietary MOS was also found to profoundly increase the goblet cell "cup" size and selectively enhanced the differentiation of acidic and sulphated-acidic mucins goblet cells.

In Chapter 4, the possible interrelationship between gut microflora and intestinal mucins is presented. The modulating effects of MOS and zinc bacitracin (ZnB) on gut microbial composition as revealed by a combination of the conventional plate culture method and a molecular technique, Terminal-restriction fragment length polymorphisim (T-RFLP), were also discussed. Dietary MOS promoted the growth of lactobacilli in the ileal mucosa and *vice versa* for ileal and caecal clostridia. As opposed to this, ZnB suppressed the growth of intestinal bacteria. Use of T-RFLP further revealed that MOS increased the diversity of

lactobacilli in the ileal digesta and ileal mucosa, whereas ZnB suppressed their diversity. However, both MOS and ZnB significantly increased the proportional abundance of ileal lactobacilli at the expense of the clostridia. Additionally, an apparent differential selection for certain *Lactobacillus* species by MOS and ZnB was also noted. It was also observed that supplementation of MOS or ZnB increased the homogeneity of gut microflora. Based on these results, a few possible target mechanisms identified for future selection of effective alternatives to antibiotics were discussed.

Chapter 5 highlights the main effects of MOS and dietary threonine level as well as their interactions on nutrient utilisation. No interactive effect of MOS and dietary threonine on ileal nutrient uptake and amino acid digestibility was detected. However, supplementation of MOS and adequate feeding of threonine profoundly increased the uptake of L-threonine and D-glucose as well as the apparent and standardised ileal digestibility of threonine. There was also a tendency for MOS to improve the standardised ileal digestibility of lysine. Both MOS and dietary threonine also increased the ileal flow of crude mucins. The impact of this increased ileal flow of mucins on the utilisation of nutrients was discussed.

The effects of MOS and dietary threonine on feed passage rate were investigated in Chapter 6. It was demonstrated that MOS interacted with dietary threonine to reduce the ileal mean retention time with shortest transit time noted in MOS-supplemented birds fed with adequate level of dietary threonine. Correspondingly, a tendency for a reduction in the total tract mean retention time by MOS and dietary threonine interaction was also recorded. Although not conclusive, the influential roles of intestinal mucins, volatile fatty acids, gut microflora and voluntary feed intake on feed passage rate were discussed. No effects of intestinal musculature on feed passage rate were observed.

Chapter 7 discusses the major findings on the interactive effects of dietary threonine and MOS on growth performance and the related intestinal physiological responses. The results of these studies provided evidence that MOS is a viable growth promotant for broiler chickens. As the growth-promoting effect of MOS is closely related to its modulating effects on mucosal development, gut microflora and intestinal mucin dynamics, optimisation of dietary threonine in conjunction with MOS may help to improve the efficacy of the latter. Recommendations for future studies and justification of the experimental objectives and methodologies were also discussed.