REDUCING THE PROTEIN CONTENT IN DIETS FOR WEANER PIGS TO CONTROL POST-WEANING DIARRHOEA: PHYSIOLOGICAL AND METABOLIC RESPONSES OF THE GASTROINTESTINAL TRACT

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This thesis is presented for the degree of Doctor of Philosophy of Murdoch University

June 2010
Declaration

I declare that this thesis is my own account of my research and contains as its main content work, which has not previously been submitted for a degree at any tertiary education institution.

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June 2010
SUMMARY

A series of experiments was conducted with weaner pigs to examine the effects of dietary protein content, duration of feeding diets after weaning and enterotoxigenic *Escherichia coli* (ETEC) infection on physiological and metabolic responses of the gastrointestinal tract (GIT). The general hypotheses tested in this thesis were that feeding a lower protein diet supplemented with essential amino acids (AA) to maintain an ideal dietary AA pattern would: (i) reduce indices of protein fermentation in the GIT and consequently reduce the incidence of post-weaning diarrhoea (PWD), and (ii) not hinder performance responses compared with pigs fed a commercial standard diet higher in protein content.

**Experiment 1 (Chapter 3),** was conducted to examine the interactive effects of dietary protein level (251 vs. 192 g/kg CP) and addition or no addition of 2,500 ppm zinc oxide (ZnO) in pigs infected or not infected *per os* with ETEC on performance, PWD and the selected GIT measurements. The lower protein diet was formulated to achieve the ideal pattern with crystalline isoleucine (Ile) and valine (Val). The hypotheses tested were that (i) feeding a diet higher in protein in combination with ZnO would reduce the incidence of PWD in infected pigs and improve performance in comparison to pigs fed a higher protein diet without ZnO; (ii) feeding a low protein diet with ZnO would improve performance compared to feeding a low protein diet without ZnO but would have no additional effect on reducing the incidence of PWD, and (iii) feeding a lower protein diet without ZnO under ETEC challenge would reduce the incidence of PWD compared with feeding a higher protein diet with ZnO.

The major findings were:

- Infection with ETEC decreased growth (P<0.001) and feed conversion efficiency (P<0.01). Protein level had no effect on performance of pigs while ZnO supplementation increased average daily gain (ADG).
Feeding either a low protein diet (P<0.05) or ZnO supplementation (P<0.01) decreased the incidence of PWD, while infection increased the incidence of PWD (P<0.01).

Feeding a lower protein diet decreased plasma urea nitrogen (PUN) (P<0.001) and faecal ammonia-nitrogen (NH$_3$-N) (P<0.001) contents, but had no effect on the faecal volatile fatty acids (VFA) content and molar proportion of branched-chain fatty acids (BCFA) (P>0.05) compared with feeding a higher protein diet or ZnO-supplemented diets.

In non-infected pigs, feeding a lower protein diet caused a lower pH in the jejunum and ileum compared with pigs fed a higher protein diet (P<0.05 and P<0.01, respectively). However, feeding ZnO-supplemented diets increased (P<0.05) pH in the stomach and caecum compared with feeding diets without ZnO supplementation.

Two- and three-way interactions between the independent variables were not evident (P>0.05) for performance and the incidence of PWD, indicating that feeding a lower protein diet and supplementation of ZnO are not additive and either strategy could be used independently as a dietary intervention for reducing PWD at weaning.

**Experiment 2 (Chapter 4),** examined the effects of feeding duration of a lower protein diet (173 g/kg CP) or a higher protein (243 g/kg CP) on the incidence of PWD, gastrointestinal protein fermentation indices and performance after weaning in pigs were not infected, in order to make it significant to the next experiment (Chapter 5). The lower protein diet was formulated to achieve the ideal pattern with crystalline Ile and Val. The hypotheses tested in the study were that feeding a diet lower in protein but supplemented with essential AA to maintain an ideal AA pattern for 7 to 10 days after weaning would: (i) reduce indices of protein fermentation in the GIT and reduce the incidence of PWD, and (ii) not compromise growth in the post-weaning period.

The major findings were:
• Feeding a lower protein diet, regardless of duration of feeding, decreased PUN (P<0.001) and faecal NH$_3$-N (P<0.001) contents.

• Feeding a lower protein diet, irrespective of feeding duration, decreased the incidence of PWD at day 8 after weaning (P<0.05), and caused firmer faeces (P<0.05) between days 10 and 12 after weaning compared with feeding a higher protein diet.

• Total-tract apparent digestibility of dry matter (DM), energy and crude protein was similar between treatments, and feeding a lower protein diet did not compromise performance of pigs up to 106 days after weaning.

**Experiment 3 (Chapter 5)**, examined the effect of feeding low protein (175 g/kg CP) diets for 7 or 14 days after weaning or a high protein (256 g/kg CP) diet for 14 days after weaning on the incidence of PWD, indices of protein fermentation and production in pigs with or without infected with ETEC. The lower protein diet was formulated to achieve the ideal pattern with crystalline Ile and Val. The hypothesis tested in this study was that feeding a low protein diet would decrease the incidence of PWD without compromising growth. To elucidate whether bacterial infection pressure interacts with dietary protein level, an experimental ETEC infection model established in this context.

The major findings were:

• Feeding a lower protein diet decreased PUN (P<0.001) and faecal NH$_3$-N (P<0.001) contents indicating reduced protein fermentation in the GIT of the pigs fed a lower protein diet compared with pigs fed a higher protein diet after weaning.

• Feeding a lower protein diet decreased the incidence of PWD (P<0.001), and increased faecal DM content (P<0.001), compared with pigs fed a higher protein diet after weaning.

• Infection increased the shedding of β-haemolytic *Esherichia coli* (*E. coli*) (P<0.001), the incidence of PWD (P<0.001) and faecal NH$_3$-N contents (P<0.01), but did not interact with dietary protein level after weaning.

• The interaction between dietary protein level and ETEC infection was not significant for production, however pigs challenged with ETEC grew
slower (P<0.001) and had poorer feed conversion efficiency (P<0.01) compared with non-ETEC infected pigs after weaning.

- My study showed that the reduction in PWD was associated with decreased protein fermentation indices in the distal GIT, and that using the ETEC challenge model (in contrast to Experiment 2) caused a much greater incidence of PWD. Otherwise, the results would be less clear or results obtained might be difficult to translate to commercial practice. Furthermore, I showed that a low protein diet only needs to be fed for 7 days after weaning to achieve its positive effects.

**Experiment 4 (Chapter 6)**, was conducted in response to Experiment 3, and evaluated in more detail how a low protein diet (190 g/kg CP) might reduce PWD compared to feeding a high protein diet (239 g/kg CP) in pigs infected or not infected *per os* with ETEC. Pigs were euthanased at 7 or 14 days after weaning as these were considered critical time points in the post-weaning processes likely to influence PWD. The low protein diet was formulated to achieve the ideal pattern of AA with crystalline Ile and Val. The hypotheses tested were that feeding a low protein diet would (i) maintain comparable nitrogen (N) digestibility at the terminal ileum, and hence decrease intestinal flow of dietary origin-N, and (ii) decrease protein fermentation indices in the large intestine, and hence reduce the risk of the PWD.

The major findings were:

- Consistent with previous studies, PUN and faecal NH$_3$-N levels were lower (P<0.001) after a lower protein diet, concomitant with decreased nitrogen content in the ileum plus all sites of the large intestine (P<0.05 - P<0.001), irrespective of ETEC infection.

- Feeding the low protein diet decreased (P<0.001) total N intake and ileal dietary-origin N flow but did not alter (P>0.05) the apparent ileal digestibility (AID) of N at either 7 or 14 days after weaning. Although ETEC infection decreased (P<0.05) overall the AID of nitrogen, feeding the low protein diet consistently reduced ileal N flow.

- Feeding a low protein diet decreased pH at the jejunum and ileum
(P<0.05 and P<0.01, respectively), while ETEC infection increased pH at the distal colon (P<0.05).

- Feeding the high protein diet increased (P<0.05) the incidence of PWD, and ETEC infection increased (P<0.01) the incidence of PWD only in pigs fed a high protein diet. PWD was more prevalent at day 7 after weaning (infection × feeding durations interactions, P<0.05).
- ETEC infection decreased (P<0.05) daily gain and feed conversion efficiency but feeding a lower protein diet for 14 days after weaning did not compromise production efficiency even in the ETEC infected group.

**Experiment 5 (Chapter 7)**, was a preliminary study aimed at determining the influences of dietary protein level (239 g/kg vs. 190 g/kg CP), ETEC infection and feeding duration after weaning on selected bacterial populations in the GIT of newly-weaned pigs, using terminal restriction fragment length polymorphism (T-RFLP). The purpose of this study was to provide some preliminary data in relation to the possible effects of protein content and ETEC infection on microbial diversity in the GIT of weaner pigs.

The major findings were:

- Dietary protein levels had no effect on the relative abundance of selected microbiota (P>0.05) in all sites of the large intestine.
- ETEC infection increased the relative abundance of *E. coli* [370 base pair (bp)] in the caecum (P<0.05), proximal (P<0.05), and distal colon (P<0.01).
- ETEC infection also increased the relative abundance of *Pseudomonas aeruginosa* (151 bp) in the caecum (P<0.01), and by tendency in the proximal (P<0.1) and distal colon (P<0.1).
- ETEC infection tended to decrease the relative abundance of *Megasphaera elsdenii* (588 bp) in the caecum (P<0.1).
- The relative abundance of *Pseudomonas aeruginosa* tended to decrease in the proximal and distal colon (P<0.1), but the relative abundance of *Megasphaera elsdenii* was increased (P<0.05) in pigs fed the diets for 14 days after weaning compared with pigs fed the diets for 7 days (P<0.1).
• In the frequency analysis, dietary protein levels, ETEC infection and feeding duration after weaning had no effect on total number of detected peaks (P>0.05) in the all sites of the large intestine (P>0.05).
• ETEC infection increased (P<0.1 - P<0.05) the frequency of Pseudomonas aeruginosa and E. coli in the all sites of large intestine, and tended to increase Proteus mirabilis (371 bp) and Lactobacillus acidophilus (593 bp) (P<0.1) in the caecum.
• The results suggested that even though reduced dietary protein level decreased protein fermentation indices, decreased protein content in the dietary chyme did not alter the abundance and frequency of the selected members of the microbiota, while infection with ETEC did.

From the results obtained in this thesis, I propose that:
• Feeding a lower protein diet, supplemented with crystalline AA to conform to the recommended ideal AA pattern, for only 7 days after weaning reduces the incidence of PWD commensurate with reductions in selected protein fermentation indices indicative of enhanced gut health, and without compromising the overall production efficiency of pigs. Feeding a low protein diet or adding 2,500 ppm ZnO to either a high or low protein diet reduces the incidence of PWD, in particular under ETEC infection pressure. Therefore either feeding a high protein diet with ZnO supplementation or feeding a low protein diet supplemented with AA but not ZnO can be used as dietary strategies to minimise PWD.
• Low protein diets most likely reduce the incidence of PWD by restricting the amount of N that enters the lower sections of the GIT of the newly-weaned pig. A reduction in the amount of N, from both exogenous and endogenous sources, reduces the extent of the formation of compounds such as NH₃, which have been linked to the development of PWD.
• Feeding a lower protein diet can be a part of an alternative strategy to the use of antimicrobial agents in the diet, which is suitable for commercial production systems where pigs are exposed to high levels of ETEC challenge and there are restrictions, legislative or otherwise, on the use of antimicrobials in diets.
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CHAPTER 3
EFFECTS OF DIETARY PROTEIN LEVEL AND ZINC OXIDE
Acknowledgements

I would like to extend thanks and appreciation from my deepest heart to my principal supervisor Dr John R. Pluske for giving me the opportunity to work on this challenging area of research and towards my degree. Without his clear supervision, mentorship, moral support and encouragement, this thesis would not have conducted. My sincerest thanks extend to my co-supervisor Dr Jae C. Kim. He motivated me to enrich my knowledge of digestive physiology and nutrition of pigs. I thank Dr Jae C. Kim again for his assistance, enthusiastic support, patient and extensive knowledge, and also for his friendship and leadership. The same gratitude is addressed to Dr David J. Hampson for his help and unconditional support. I also wish to thank Dr Bruce P. Mullan at Department of Agriculture and Food for his supports for formulating the experimental diets throughout this study, and also for permissions to use the great research facility at Median Research Station. I also would like to thank Dr Christian F. Hansen for his encouragement and support. I am also appreciative of technical support from Mr Nicholls Roland at Department of Agriculture and Food. It was my honour to work with them.

My thanks are extended to Ms Josie Mansfield for her technical expertises. Many thank Josie. Appreciative thank is also to Ms Aracely Hernandez and my colleague Ms Danka Halas for their help and support throughout this study. Thanks are extended to Mr Richard Seaward, Bob Davis, and other staff at Medina Research Station for their unlimited technical support during the animal experiments.

I wish to acknowledge an Australian Research Council Linkage Grant, Danish Pig Production and Evonik Degussa for their financial support and kind donation, respectively. I was the grateful recipient of a Murdoch International Post-Graduate Award from Murdoch University, and thank Murdoch University for the support during this PhD programme.

Last but not least, my deepest thanks are extended to my wife Kyong-Mi Lee for
her endless love, unlimited support and encouragement. I am also grateful for the same supports of my parents and my parents in law. I thank you all for the great concerns and the help.
Publications and conference paper

Journal articles

Halas, D., J. M. Heo\textsuperscript{A}, C. F. Hansen, J. C. Kim, D. J. Hampson, B. P. Mullan, and J. R. Pluske. 2007. Organic acids, prebiotics and protein level as dietary tools to control the weaning transition and reduce post-weaning diarrhoea in piglets. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 2, No. 079: 13. (Chapter 1)


Heo, J. M.\textsuperscript{B}, J. C. Kim, C. F. Hansen, B. P. Mullan, D. J. Hampson, and J. R. Pluske. Effects of dietary protein level and zinc oxide supplementation on performance responses and gastrointestinal tract characteristics in weaner pigs challenged with an enterotoxigenic strain of \textit{Escherichia coli}. Animal Production Science (Submitted) (Chapter 3)


Heo, J. M., J. C. Kim, C. F. Hansen, B. P. Mullan, D. J. Hampson, and J. R. Pluske. 2009. Feeding a diet with decreased protein content reduces indices of protein fermentation and the incidence of postweaning diarrhea

\textsuperscript{A} Equally contributed
\textsuperscript{B} Published in part
in weaned pigs challenged with an enterotoxigenic strain of *Escherichia coli*. Journal of Animal Science. 87: 2833-2843 (Chapter 5)

**Heo, J. M.,** J. C. Kim, C. F. Hansen, B. P. Mullan, D. J. Hampson, and J. R. Pluske. Feeding a diet with a reduced protein content reduces nitrogen content in the gastrointestinal tract and post-weaning diarrhoea, but does not affect apparent nitrogen digestibility, in pigs challenged with an enterotoxigenic strain of *Escherichia coli*. Animal Feed Science and Technology. (Accepted for publication) (Chapter 6)

**Refereed conference proceedings**


**Heo, J. M.,** J. C. Kim, B. P. Mullan, C. F. Hansen, D. J. Hampson, and J. R. Pluske. 2009. Feeding a lower protein diet reduces nitrogen content in the

**Non-refereed conference proceedings**

Abbreviations used in this thesis:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AA</td>
<td>amino acid(s)</td>
</tr>
<tr>
<td>ADF</td>
<td>acid detergent fibre</td>
</tr>
<tr>
<td>ADG</td>
<td>average daily gain</td>
</tr>
<tr>
<td>ADFI</td>
<td>average daily feed intake</td>
</tr>
<tr>
<td>Ammonia nitrogen</td>
<td>NH$_3$-N</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>BCFA</td>
<td>branched-chain fatty acids</td>
</tr>
<tr>
<td>BW</td>
<td>body weight</td>
</tr>
<tr>
<td>DE</td>
<td>digestible energy</td>
</tr>
<tr>
<td>DM</td>
<td>dry matter</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>E. coli</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>ETEC</td>
<td>enterotoxigenic Escherichia coli</td>
</tr>
<tr>
<td>FCR</td>
<td>feed conversion ratio</td>
</tr>
<tr>
<td>GE</td>
<td>gross energy</td>
</tr>
<tr>
<td>GIT</td>
<td>gastrointestinal tract</td>
</tr>
<tr>
<td>GLM</td>
<td>general linear model</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>ME</td>
<td>metabolisable energy</td>
</tr>
<tr>
<td>N</td>
<td>nitrogen</td>
</tr>
<tr>
<td>NDF</td>
<td>neutral detergent fibre</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>PUN</td>
<td>plasma urea nitrogen</td>
</tr>
<tr>
<td>PWD</td>
<td>post-weaning diarrhoea</td>
</tr>
<tr>
<td>r</td>
<td>correlation coefficient</td>
</tr>
<tr>
<td>VFA</td>
<td>volatile fatty acids</td>
</tr>
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Three-letter abbreviations for AA (e.g., Ala) is used in the current thesis