FACTORS INFLUENCING THE TOTAL TRACT APPARENT DIGESTIBILITY OF NUTRIENTS FROM WHEAT FOR THE WEANLING PIG

Jae Cheol Kim

BSc (Agric), MSc (Nutritional Science)

Division of Health Science,
School of Veterinary and Biomedical Sciences,
Murdoch University

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

July 2003

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.

Jae Cheol Kim

July 2003

SUMMARY

The objectives of the studies conducted in the Part A of this thesis were to quantify the variation of digestible energy (DE) content of wheat and to document the responsible factors for the variation. The general hypothesis tested in Part A of this thesis was that the variety, growing region, growing season, supplementation of xylanase and post-harvest grain storage (i.e., six months of storage at ambient temperature) will influence the DE content of wheat when fed to weaner pigs.

Experiment 1 (Chapter 3) examined the variation in the chemical composition of wheats as influenced by *variety* (Arrino, Westonia and Stiletto were selected), *growing region* (each wheat was selected from a high, medium and low rainfall region), *season* (1999 and 2000) and post-harvest *storage* for 6 months. In this experiment, up to a 19% variation in the crude protein (CP) content (mean13.3, SD 2.59), up to a 5% variation in the starch content (mean 65.4, SD 3.56), and a 10-20% variation in the total (mean 9,2, SD 0.86), insoluble (mean 8.1, SD 0.84) and soluble (mean 1.1, SD 0.23) non-starch polysaccharides (NSP) contents were observed. The major experimental findings were:

- Variety of wheat influenced the variation of most carbohydrate components, including fast digestible starch (FDS) ($P<0.001$), acid detergent fibre (ADF) ($P<0.01$), total and insoluble NSP ($P<0.05$), and *in vitro* extract viscosity ($P<0.05$);
- The annual precipitation level in each region correlated to bushel weight ($P<0.01$), protein ($P<0.01$) and carbohydrate composition, including starch ($P<0.05$), soluble NSP ($P<0.001$), lignin ($P<0.001$) and free sugars ($P<0.001$), indicating the importance of the growing environment on the DE content of wheat;
- Growing season influenced bushel weight ($P<0.001$), CP ($P<0.01$), amylose and amylopectin (components of starch, $P<0.05$), ADF ($P<0.001$), lignin ($P<0.001$), soluble NSP ($P<0.001$) and free sugars contents ($P<0.001$), and the *in vitro* extract viscosity of wheat ($P<0.05$);
- Storage of wheat decreased ADF ($P<0.05$), lignin ($P<0.01$) and soluble NSP contents ($P<0.01$) and increased the free sugar content ($P<0.001$).

In Experiment 2 (Chapter 4), the effect of variety, growing region and growing season on the DE content of wheat in 5-week-old male weaner pigs was examined, and correlations between various chemical composition of wheat and DE content were established. The major findings were:

- The DE content of wheats varied by up to 1.3 MJ/kg (12.5-13.8; mean = 13.3) in 1999 and by up to 1.8 MJ/kg (12.6-14.4; mean = 13.7) in 2000;
- Both the variety and growing region significantly influenced ($P<0.05$ - $P<0.001$) the DE content of wheat;
- Generally, the wheat variety Westonia and wheats grown in the low rainfall region contained a higher DE content than other wheats and growing regions;
- In addition, growing season influenced ($P<0.001$) DE content of wheat;
- The mean DE content determined with weaner pigs in the current study was approximately 1 MJ/kg DM lower when compared to published estimates of the DE content of wheat determined with growing pigs;
- Prediction of *in vivo* DE content of wheat for weaner pigs using NIRS (Near Infra-Red Spectroscopy) was not successful.
- Significant inverse relationships between the DE content and xylose ($P<0.05$), NDF ($P<0.01$), total-P ($P<0.01$) and phytate-P content ($P<0.05$) of wheat were seen in 1999. However, such relationships were not significant in 2000, indicating a very strong seasonal influence on both chemical composition and DE content of wheat.

Experiment 3 (Chapter 5) examined the effects of a supplemental enzyme (xylanase) and storage for 6 months on the DE content of wheats. In 1999, use of the enzyme tended to increase the DE content from 13.3 to 13.6 MJ/kg ($P=0.065$) whereas in 2000, use of the enzyme caused no change in DE content (13.7 to 13.6 MJ/kg). However, the response to enzyme addition was much greater in some wheats grown in some regions than others, suggesting that the DE response to xylanase supplementation was not systematic but appeared to be associated with the grain structure, with the DE content consequently arising from an interaction between variety and growing conditions of the wheat. For wheats harvested in 1999, storage improved the DE content from 13.3 to 14.0 MJ/kg ($P<0.01$), but for the 2000 wheats, storage for 6 months decreased DE content from 13.7 to 13.0 MJ/kg ($P<0.001$). However, the 2000 data need to be interpreted with caution because some of the wheats were infested with weevils.

The DE response to supplemental xylanase in 1999 was negatively correlated to the total and insoluble arabinose to xylose ratio ($P<0.01$), *in vitro* extract viscosity ($P<0.01$)

and lignin (P<0.06), while FDS (P<0.06) and phytate-P (P<0.06) positively correlated to the DE response to xylanase.

The DE response to storage in 1999 was positively correlated to the NDF (P<0.001) and xylose contents (P<0.01) of the wheats, which had negative influences on the DE content of non-stored wheats. The addition of xylanase in stored wheat did not improve the DE content, suggesting changes in chemical composition (eg, activation of endogenous xylanases) during storage.

In Experiment 4 (Chapter 6), the same wheats were analysed for phosphorus (P) and phytate-P content, as the latter is considered to be anti-nutritive in pig diets. The total P and phytate-P content of all wheat samples ranged 2.6 (s.e. 0.64) g/kg and 1.8 (s.e. 0.13) g/kg DM, respectively. Endogenous phytase activity was highly variable and averaged 563 (s.e. 29.6) FTU/kg between wheats. The variety, growing region, season and storage of wheat did not influence the variation of P content. However, the precipitation level over two growing seasons was positively correlated to total-P (P<0.05) and phytate-P contents (P<0.05) of wheat. Phytate-P content of wheat can be predicted from total-P content (r=0.974, P<0.001).

In Part B, two experimental designs were generated based on the results of experiments described in Part A. From the previous experiment (Experiment 2, Chapter 4), it was evident that the structure of starch (i.e., amylose: amylopectin ratio) and phytate-P content of wheat were correlated to DE content of wheat. To reinforce these established correlations the following experiments were carried out, with the general hypothesis that the structure of starch (i.e., waxy *vs.* non-waxy wheat), particle size of ground wheat, and phytate-P content of wheat will influence the digestibility of nutrients (i.e., nitrogen, energy, minerals) in wheat-based weaner pig diets.

In Experiment 5, the same variety (Janz) of a waxy (98% of total starch was amylopectin) isoline and a non-waxy isoline (71% of total starch was amylopectin) wheat were ground through a hammermill fitted either with a 8.5 mm or 4.5 mm screen to achieve average particle sizes of 930 and 560 μm, respectively. Diets were formulated and then fed to weaner pigs (3-5 weeks of age) with or without xylanase plus β-glucancase. Digestibilities of starch, nitrogen, energy, and DE content of diets were measured at day 7 and day 21 to examine any age-related improvement of nutrient

digestibilities during the first 3 weeks post-weaning. Waxy wheat improved total tract digestibility of starch (P<0.05) and CP (P<0.05), and the reduction of particle size improved total tract starch digestibility (P<0.001) at both 7 and 21 days after weaning. However, energy digestibility and DE content of the diet were not influenced by either wheat type or particle size. Supplementation of xylanase plus β-glucanase improved starch digestibility (P<0.01) and DE content (P<0.05) but not other nutrients, and this occurred especially in non-waxy (P=0.03) and larger particle-sized wheat (P=0.01). Pigs increased ability to digest protein with age (P<0.05). However, the improvement in nutrient digestibilities in waxy and fine particle size wheat diets did not translate to improved pig performance.

In Experiment 6, the hypothesis tested was that the digestibility and pig performance responses to supplemental xylanase, phytase and xylanase plus phytase would differ in weaner pigs according to P content of wheat. To test this hypothesis, two wheats containing high and low levels of total P (2.52g *vs.* 3.76 g total-P/kg DM) were obtained and fed to weaner pigs. Diets either had no enzyme or were supplemented with xylanase, phytase, or a combination of xylanase plus phytase. The hypothesis was partly supported in this experiment, since P and Ca digestibility were influenced by a wheat P content by enzyme ineraction. Overall improvements in macronutrient digestibility and pig performance by supplementation of various enzymes were not significant. Daily growth (P<0.05 – P<0.01) and FCR (P<0.05 – P<0.01) were improved by the supplemental enzyme only in the first week of the feeding trial, mainly due to the increased P and Ca availability induced by the enzymes. Amounts of P and Ca digested were below the recommended requirements by NRC in the first two weeks of the feeding trial. Supplementation of phytase generally improved P and Ca digestibility (P<0.05) in both low-P and high-P wheats. However, xylanase plus phytase supplementation did not produce synergistic effect for macronutrients, and mineral digestibilities, over single supplementation of xylanase or phytase.

From the results obtained in this thesis I propose that:

1) The DE content of wehat for weaner pigs is variable due mainly to the change of chemical composition mediated by variety, growing environment and storage after harvest, and efficacy of supplemental xylanase is dependent on the chemical structure of NSP present in cell walls of wheat;

2) Structure of starch, particle size, age after weaning, and P content of wheat are factors influencing nutrient digestibility in weaner pigs fed a wheat-based diet, and the efficacy of phytase on P and Ca digestibilities is dependent on the phytate-P content of wheat.

Table of Contents

Acknowledgement

My sincere thank from the deepest of my heart is extended to my principal supervisor Dr John Pluske for his guidance, assistance and encouragement throughout this study. Without John's enthusiastic support and extensive knowledge, this thesis would not have been possible. Many thanks John. The same gratitude is addressed to my external co-supervisor Dr Bruce Mullan at the Department of Agriculture for his assistance during the formulation of experimental diet and priceless support throughout this study. I also thank to Bruce for giving me a permission to use the great facilities at Medina Research Station. I would like to thank to Dr Darryl D'Souza at the Department of Agriculture for his endless support during the use of calorimeter. It was an honour to work with such a great people.

Appreciative thank is also due to Dr Howard Simmins at Danisco Animal Nutrition in UK for his support for analyses of wheat extract viscosity, NSP and enzyme activity, and also for valuable comments and contributions to this thesis.

I wish to thank to Dr Peter Selle at the University of Sydney in Camden for his suggestions and help for analyses of phytate-phosphorus and intrinsic phytase activity in wheat. The funding from BRI Australia for the analyses is acknowledged.

Donation of wheat samples used in this thesis by the grain farmers and Department of Agriculture is appreciated.

My thanks are extended to Dr Ru Yinjun at the South Australian Research and Development Institute (SARDI), Ms Shuyu Song at the University of New England, Armidale, and Dr David Allen at the Chemistry Centre of WA for analyses of nitrogen, gross energy, NSP and minerals. Help from Mr John Beesley at the University of Western Australia for freeze-drying samples is appreciated.

I am also grateful for the willing support and technical expertises of Dr Leanne Twomey, Mr Ken Chong, Ms Fiona Lockwood and Miss Belinda Black at the School of Veterinary and Biomedical Sciences, Murdoch University, during chemical analysis throughout this study.

Thanks are also due to: Mr John Ferguson, Mr Bob Davis, Mr Richard Seaward and other staff at Medina Research Station for their excellent technical support throughout the animal experiments.

I wish to acknowledge the Pig Industry Compensation Fund of WA and Danisco Animal Nutrition, UK, for funding this research.

I was the grateful recipient of an Australian Post-Graduate Award from Murdoch University, and thank them very much.

The companionship and advice of post-graduate students and staff members of the School of Veterinary and Biomedical Sciences is gratefully acknowledged.

I also would like to thanks to Dr Mike Taverner, Dr Ben Gursansky at Australian Pork Limited and students of Pig-Grad Network for their enthusiastic support and assistance during the last three years. The networking with other postgraduates was excellent to learn research skills and to share information about the current research interests and industry trends.

Thanks to my and my wife's family in South Korea for their love, encouragement and support during the study. I would like to dedicate this thesis to my late dad.

Finally with all my heart, I am indebted to my wife Mi Sook, son Hyun, and daughter Suha for their endless love, support and encouragement. Many thanks and I love you all.

Publications and conference abstract titles

Journal articles

Kim, J.C., Mullan, B.P., Selle, P. and Pluske, J.R. (2002). Levels of total phosphorus, phytate-phosphorus and phytase activity in three varieties of Western Australian wheats in response to growing region, growing season and storage. *Australian Journal of Agricultural Research* **53:** 1361-1366. (Chapter 6)

Kim, J.C., Mullan, B.P., Simmins, P.H. and Pluske, J.R. (2003). Variation in the chemical composition of wheats grown in Western Australia as influenced by variety, growing region, season and post-harvest storage. *Australian Journal of Agricultural Research* **54:** 541-550. (Chapter 3)

Kim, J.C., Mullan, B.P., Simmins, P.H. and Pluske, J.R. (2004). Effect of variety, growing region and growing season on digestible energy content of wheats grown in Western Australia for weaner pigs. *Animal Science* (In Press, Chapter 4)

Kim, J.C., Mullan, B.P., Simmins, P.H. and Pluske, J.R. (2004). Wheat phosphorus content and supplemental enzymes determine responses in nutrient digestibility and growth performance of weaner pigs. *Animal Feed Science and Technology* (Submitted, Chapter 8)

Conference proceedings

Kim, J.C., Mullan, B.P. and Pluske, J.R. (2001). Storage influences the protein and carbohydrate composition of wheat. In: *Recent Advances in Animal Nutrition in Australia* **13**. (Ed, J.L. Corbett), p. 34A. The University of New England: Armidale, NSW.

Kim, J.C., Mullan, B.P., Henryon, M.A., Simmins, P.H. and Pluske, J.R. (2001). Variety, growing region and addition of exogenous xylanase influences digestible energy content of wheat fed to weaner pigs. In: *Manipulating Pig Production VIII*. (Ed, P.D.Cranwell), p.219. Australasian Pig Science Association: Werribee.

Kim, J.C., Mullan, B.P., Simmins, P.H. and Pluske, J.R. (2001). Relationship between the quantity and quality of carbohydrates and the digestible energy content of wheats for weaner pigs. In: *Manipulating Pig Production VIII*. (Ed, P.D.Cranwell), p.203. Australasian Pig Science Association: Werribee.

Kim, J.C., Mullan, B.P., Simmins, P.H. and Pluske, J.R. (2001). Can a change in starch content following storage predict the change in digestible energy content of wheat fed to weaner pigs? In:

Manipulating Pig Production VIII. (Ed, P.D.Cranwell), p.204. Australasian Pig Science Association: Werribee.

Kim, J.C., Mullan, B.P., Simmins, P.H. and Pluske, J.R. (2003). Xylanase supplementation to increase DE content in wheat-based diets for weaner pigs is influenced by variety and growing region. In: *Proceedings of 9th International Symposium on Digestive Physiology in Pigs, Banff, Alberta, Canada, May 2003.* (Ed, R.O. Ball), pp. 299-301. Department of Agricultural, Food and Nutritional Science, University of Alberta, Canada.

Kim, J.C., Mullan, B.P. and Pluske, J.R. (2003). Nutrient digestibility of wheat for weaner pigs depends on starch structure, particle size and enzyme. In: *Manipulating Pig Production IX*. (Ed, J.E. Paterson), p. 41. Australasian Pig Science Association: Werribee.

Kim, J.C., Simmins, P.H., Mullan, B.P. and Pluske, J.R. (2003). Interactive effects of wheat phosphorus content and enzymes on mineral digestibility in weaner pigs. In: *Manipulating Pig Production IX*. (Ed, J.E. Paterson), p.165. Australasian Pig Science Association: Werribee.

Book Chapter

Pluske, J.R., **J.C. Kim**, D.E. McDonald, D.W. Pethick and D.J. Hampson (2001). Non-starch polysaccharides in the diet of young weaned piglets. In: The weaner pig: nutrition and management. (Eds; M.A. Varley, J. Wiseman), pp. 81-112. CABI Publishing, U.K.

Abbreviations used in this thesis:

AA:	amino acids
ADF:	acid detergent fibre
ADG:	average daily gain
Amy/Ap:	Amylose to amylopectin ratio
ANF's:	anti-nutritional factors
ANOVA:	analysis of variance
Ara/Xyl:	Arabinose to xylose ratio
APW:	Australian premium white
ASWN:	Australian standard white noodle
CP:	crude protein
DC:	digestibility coefficient
DCe:	digestibility coefficient of energy
DE:	digestible energy
DF:	dietary fibre
DM:	dry matter
DMSO:	dimethyl sulphoxide
DW:	distilled water
FCR:	feed conversion ratio
FDS:	fast digestible starch
GE:	gross energy
GIT:	gastrointestinal tract
GLM:	general linear model
ME:	metabolisable energy
N:	nitrogen
NDF:	neutral detergent fibre
NIR:	near infra-red spectroscopy
NS:	not statistically significant (P>0.05)
NSP:	non-starch polysaccharides
TNSP:	total non-starch polysaccharides
INSP:	insoluble non-starch polysaccharides
SNSP:	soluble non-starch polysaccharides
P:	phosphorus
r:	correlation coefficient