FEED ENZYMES AND WHOLE WHEAT IN POULTRY DIETS

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SUPERVISOR'S DECLARATION

This is to certify that the research carried out for the Doctoral thesis entitled: " Feed Enzymes and Whole Wheat in Poultry Diets" was done by Yuben Wu in the Institute of Food, Nutrition and Human Health at Massey University, Palmerston North, New Zealand. The thesis material has not been used in part or in whole for any other qualification, and I confirm that the candidate has pursued the course of study in accordance with the requirements of the Massey University regulations.

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CANDIDATE'S DECLARATION

This is to certify that the research carried out for my Doctoral thesis entitled: " Feed Enzymes and Whole Wheat in Poultry Diets" in the Institute of Food, Nutrition and Human Health at Massey University, Palmerston North, New Zealand is my own work and that the thesis material has not been used in part or in whole for any other qualification.

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ABSTRACT

Seven studies were undertaken to examine the effects of microbial phytase, glycanases, and whole wheat feeding in broiler diets. The major focus of this doctoral research was to investigate the effects of a microbial phytase produced by solid state fermentation in broiler diets.

1. The aim of the first study was to examine the effects of microbial phytase on the performance, apparent ileal digestibility of phosphorus (P), phytate P and nitrogen, and utilisation of nutrients in male and female broilers fed wheat-soy diets from 1 to 42 day of age. There were eight dietary treatments. Diets 1 to 4 were supplemented with inorganic phosphorus to contain 0.30, 0.36, 0.42 and 0.48% of non-phytate P (nP), respectively in the starter phase (1-21 day) and 0.20, 0.26, 0.32 and 0.48% in the finisher phase (22-42 day), respectively. Diets 5 to 8 were based on diet 1 and supplemented with phytase to contain 500, 1000, 1500 and 2000 PU/kg diet, respectively. Within sex, each of the eight dietary treatments was assigned to five pens of eight birds each. In both sexes, weight gain (P<0.05 to 0.001), feed efficiency (P<0.05 to 0.001) and toe ash contents (P<0.001) were increased as the P or phytase were added to low-P diet (diet 1). The magnitude of increments in performance and toe ash contents parameters were greatest with the first addition of P or phytase and then tended to plateau with further additions. Feed efficiency of birds fed phytase-supplemented diets was superior to those fed adequate-P diets. Toe ash contents of birds fed low-P diet with 500 PU/kg diet were comparable to those fed adequate-P diets. Addition of 500 PU/kg phytase to the low-P diet increased the nitrogen digestibility by 3.1 and 5.3% in males and females, respectively. Addition of phytase increased the apparent metabolisable energy (AME) of wheat-soy diets during the starter and finisher phases, but the increments were greater in the finisher diets. Based on weight gain responses to graded additions of supplemental non-phytate P and phytase, estimates were obtained for P equivalency of the microbial phytase. These estimates translate into P release values of 72 to 131% from phytate and are apparently spurious. These unexpectedly high equivalency estimates may be due, partly, to the secondary enzymes activities present in the phytase product evaluated.

2. Selected data from the first study were analysed to compare the influence of sex on the performance, toe ash contents, phytate P release, AME and digestibility of nutrient

in broilers fed diets containing low and adequate dietary levels of phosphorus. Sex of broilers had no effect on AME values determined during week 3. During week 6, the AME values for male broilers were higher ($P<0.01$) than those for the females. Female broilers tended ($P<0.10$) have a higher ileal nitrogen digestibility than the males. Apparent ileal phytate-P degradation values in males were higher than those in females (0.282 vs 0.234), but the differences were not significant ($P>0.05$). A significant interaction ($P<0.05$) between nP level x sex was observed for apparent ileal P digestibility. Increasing dietary nP levels increased apparent ileal P digestibility in both males and females, but the improvements were higher in females (13.4 vs 6.1 percentage units).

3. The aim of the second study was to examine the influence of microbial phytase addition on the performance, toe ash contents and nutrient utilisation of male broilers fed diets based on corn and wheat. The experiment was conducted as a 2 x 2 x 2 factorial arrangement of treatments. Within the factorial, two diet types (corn-soy or wheat-soy) containing two levels of non-phytate P (0.30 or 0.45%) were evaluated and each level of non-phytate P was supplemented with 0 or 500 PU phytase/kg diet. The results showed that microbial phytase was effective in both corn-based and wheat-based diets and that with supplemental 500 PU phytase/kg, dietary P level can be lowered by 0.15% to reduce excreta P output by 35% and still maintain comparable growth performance and bone mineralisation to birds fed a diet containing adequate levels of P. Phytase addition improved the AME values of wheat-based diets, but had little effect on the AME of corn-based diets. Phytase improved ileal nitrogen digestibility in both diet types, but the responses to added phytase tended to be higher in wheat-based diets, as shown by a diet type x phytase interaction ($P<0.10$).

4. The aim of the third study was to examine the influence of phytase and glycanases, individually or in combination, on the AME and nutrient digestibility of sorghum, corn, wheat and barley using 4-week-old broilers. Microbial phytase improved ($P<0.05$) apparent ileal phosphorus digestibility in all cereals. Phytase supplementation improved ($P<0.05$) the AME of corn and barley, and numerically improved the AME in sorghum and wheat. Further improvements ($P<0.05$) in the AME of wheat and barley were observed when the phytase was combined with glycanases. The observed improvements in AME were not always associated with enhanced digestibility of protein and starch.

5. In the fourth experiment, potential beneficial effects from the side activities present in

a microbial phytase produced by the solid state fermentation were examined by comparing the release of phosphorus, reducing sugars and α-amino nitrogen by two other phytase preparations in wheat- and corn-based diets using an *in vitro* digestion model. Microbial phytase produced by solid state fermentation released more ($P<0.05$) phytate-bound P (11.0% and 7.8% in wheat- and corn-based diets, respectively) and α-amino nitrogen (1.7% and 6.2% for wheat- and corn-based diets, respectively) than a phytase produced by submerged liquid fermentation without detectable side activities. Phytase produced by solid state fermentation also released 2.9% more reducing sugars in wheat-based diets and 6.2% α-amino nitrogen in corn-based diets. The superiority of microbial phytase produced by solid state fermentation in releasing nutrients in both types of diets is likely to be due to the presence of other enzyme activities.

6. In the fifth experiment, the influence of microbial phytase and xylanase, individually or in combination, on the performance, AME, digesta viscosity, digestive tract measurements and gut morphology in broilers fed wheat-soy diets containing adequate P levels were examined. The experimental diets were formulated by supplementing the basal diet with xylanase (1000 XU/kg), phytase (500 PU/kg) or combination of phytase and xylanase. The results showed that microbial phytase was as effective as xylanase in improving the performance of broilers. This may be due to the phytase product used in the study was produced by solid state fermentation and contained relatively high levels of β-glucanase, xylanase and protease. Supplemental phytase improved ($P<0.05$) the weight gains and feed efficiency by 17.5 and 2.9%, respectively. Corresponding improvements due to the addition of xylanase were 16.5 and 4.9%, respectively. Combination of phytase and xylanase had no further effects. The improved performance by supplemental phytase or xylanase was associated with reduced digesta viscosity, improved AME, and reduced relative weight and length of small intestine. Phytase and xylanase supplementation had no effect ($P>0.05$) on villus height, crypt depth, goblet cell number, epithelium thickness, and ratio of crypt depth to villus height in duodenum, jejunum and ileum. The only exception was that addition of phytase increased ($P<0.05$) villus height in the duodenum and decreased ($P<0.05$) the number of goblet cells in the jejunum compared to those in the unsupplemented basal diet. Interestingly, xylanase supplementation tended ($P<0.10$) to increase goblet cell numbers in the duodenum and decreased ($P<0.05$) crypt depth in the jejunum.

7. Whole grain feeding for broilers has received attention in recent years due to

associated economic benefits. The aim of the sixth experiment was to examine the influence of method of whole wheat inclusion and xylanase supplementation on the performance, apparent metabolisable energy, digesta viscosity, and digestive tract measurements of broilers fed wheat-based diets. A 3 x 2 of factorial arrangement of treatments was used with three diet forms (64.8% ground wheat [GW], GW replaced with 20% of whole wheat before [WW1] or after cold-pelleting [WW2]) and two enzyme doses (0 and 1000 XU/kg diet). The results demonstrated the beneficial effects of whole wheat inclusion and xylanase supplementation in broiler diets. Birds fed diets containing whole wheat had improved (P<0.05 to 0.001) weight gain (2.1-3.9%), feed efficiency (4.1-5.8%) and AME (3.6-6.0%) compared to those fed diets containing ground wheat. The relative gizzard weights of birds fed WW2 diets were higher (P<0.05) than those fed GW and WW1 diets. Pre-pelleting inclusion of whole wheat had no effect (P>0.05) on the relative gizzard weights. Post-pelleting inclusion of whole wheat resulted in greater improvements (P<0.05 to 0.001) in feed efficiency, AME and relative gizzard weights compared to the pre-pelleting treatment. Improved performance with post-pelleting inclusion of whole wheat was probably due to the development of gizzard and to improved AME. However, it is difficult to propose a mechanism for the improvements observed with pre-pelleting inclusion of whole wheat. Improvements in bird performance by xylanase supplementation were associated with reduced digesta viscosity and improved AME. Neither xylanase supplementation nor whole wheat inclusion influenced (P>0.05) the relative weights and length of the duodenum, jejunum, ileum or total small intestine.

8. The aim of the seventh study was to examine the influence of post-pelleting inclusion of whole wheat and xylanase supplementation on the performance, digestive tract measurements and carcass characteristics of broilers fed wheat-soy diets from 1 to 35 days of age. There were five dietary treatments. Diet 1 was based on corn and soybean meal. Diets 2 and 3 were based on ground wheat (GW) and soybean meal without and with added xylanase at a level of 1000 XU/kg, respectively. Diets 4 and 5 were whole wheat (WW) replacing GW (10 and 20% whole wheat replacing GW during 1-21 and 22-35 day, respectively) without and with added xylanase at a level of 1000 XU/kg, respectively. Post-pelleting inclusion of whole wheat reduced (P<0.10) weight gains, but improved (P<0.05) the feed efficiency over the 35-day experimental period. Improved feed efficiency with whole wheat inclusion was associated with the

development of gizzard. Xylanase supplementation improved the performance of broilers fed both ground wheat and whole wheat diets. Interestingly, feed efficiency of birds fed diets with whole wheat and supplemental xylanase were comparable to those fed corn-based diets. The 'apparent additivity of the combination of whole wheat and xylanase suggests that the mechanisms involved are different. Neither whole wheat inclusion nor xylanase supplementation influenced ($P>0.05$) the relative weight and length of the small intestine, carcass recovery and relative weights of breast muscle and abdominal fat pad.

PUBLICATIONS

Studies completed during candidature, some of which are reported in this thesis have been presented in the following conference proceedings:

Ravindran, V.; Wu, Y.B.; Thomas, D.V.; Camden, B.J.; Morel, P.C.H. and Hendriks, W.H. (2001) Improving phosphorus availability in broiler diets based on wheat-soybean meal using microbial phytase produced in solid state fermentation. In: *Biotechnology in the Feed Industry, Proceedings of Alltech's 17th Annual Symposium* (Lyons, T.P. and Jacques, K.A.; editors), pp. 461-490. Nottingham University Press, Nottingham, United Kingdom.

Wu, Y.B.; Ravindran, V.; Thomas, D.V.; Camden, B.J; Morel, P.C.H.; Hendriks, W.H. and Pierce, J. (2001) Efficacy of Allzyme phytase produced by solid-state fermentation in improving the phosphorus availability of wheat-soybean meal diets for broilers. *Poultry Science* **80**: (suppl. 1): 476- 477.

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Wu, Y.B.; Ravindran, V.; Thomas, D.V.; Camden, B.J.; Morel, P.C.H. and Hendriks, W.H. (2002) Broiler response to microbial phytase as influenced by diet type and dietary non-phytate phosphorus levels. *Proceedings of the Australian Poultry Science Symposium* **14**: 145-147.

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Enhanced the feed value of cereals by enzyme supplementation. *Proceedings of the 7th WPSA Asian Pacific Federation Conference*, pp. 305-308. 12th Australian Poultry and Feed Convention, Gold Coast, Queensland, Australia.

Wu, Y.B.; Ravindran, V. and Hendriks, W.H. (2003) Influence of xylanase supplementation and whole wheat inclusion on the performance and gizzard weights in broilers. *Proceedings of the Australian Poultry Science Symposium* **15**: 103.

Wu, Y.B.; Pierce, J.; Hendriks, W.H. and Ravindran, V. (2003) Comparison of *in vitro* nutrient release by three enzyme preparations in wheat- and maize-based diets. *Proceedings of the Australian Poultry Science Symposium* **15**: 114-118.

LIST OF ABBREVIATIONS

AME	Apparent metabolisable energy
AMEn	Nitrogen-corrected apparent metabolisable energy
AIND	Apparent ileal nitrogen digestibility
AIPD	Apparent ileal phosphorus digestibility
AISD	Apparent ileal starch digestibility
Arg	Arginine
BW	Body weight
Ca	Calcium
CB	Corn-based diet
Co	Cobalt
Cu	Copper
Cys	Cysteine
DCP	Dicalcium phosphate
DFP	Defluorinated phosphate
DM	Dry matter
EGP	Egg production
EP	Experimental period
FCR	Feed conversion ratio
Fe	Iron
GE	Gross energy
GMD	Geometric mean diameter
His	Histidine
Hrs	Hours
I	Iodine
Ile	Isoleucine
Lys	Lysine
Leu	Leucine
Mg	Magnesium
MCP	Monocalcium phosphate
Met	Methinonine
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
nP	Non-phytate phosphorus
NSP	Non-starch polysaccharides
P	Phosphorus
Phe	Phenylalanine
SAA	Sulphur amino acids
Se	Selenium
Ser	Serine
SSF	Solid state fermentation
1,25-$(OH)_2D_3$	1,25-dihydroxycholecalciferol
Thre	Threonine
Val	Valine
WB	Wheat-based diet
Zn	Zinc

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