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Effect of Exogenous Xylanase Supplementation on the Performance, Net Energy and Gut Microflora of Broiler Chickens Fed Wheat-based Diets

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ABSTRACT : An experiment was carried out to assess the effects of xylanase supplementation on the performance, net energy and gut microflora of broilers fed a wheat-based diet. Day-old male broiler chicks were allocated to two dietary treatments. Each treatment was composed of six replicate cages of seven broilers per cage. The diets were wheat-based and offered as mash. The treatments included i) basal diet deficient in metabolizable energy; and ii) basal diet supplemented with a commercial xylanase added at 4,000 U/kg feed. Bird performance, nutrient utilization and gut microbial populations were measured. Heat production and net energy were determined using an auto-control, open circuit respiration calorimetry apparatus. Results showed that exogenous xylanase supplementation improved feed conversion efficiency ($p<0.05$) and increased diet AME (+4.2%; $p<0.05$), as well as heat production (HP), net energy for production (NEp), production of CO_2, and consumption of O_2. The respiratory quotient (RQ) was also increased ($p<0.01$) by the addition of xylanase. NEp value was increased by 26.1% while daily heat production per kg metabolizable body weight was decreased by 26.2% when the xylanase was added. Xylanase supplementation numerically increased the ileal digestibility of protein and energy by 3 and 6 percentage units respectively ($p>0.05$). The ileal digestibility of hemicellulose was significantly improved by xylanase addition ($p<0.05$). (**Key Words :** Xylanase, Wheat, Performance, Net Energy, Gut Microflora, Broiler)

INTRODUCTION

Wheat is one of the most commonly used cereal grains in poultry feeds. However wheat contains significant amounts of soluble non-starch polysaccharides (sNSP). High levels of sNSP can result in increased viscosity in the small intestine of chickens, and depress nutrient utilization and performance (Choct and Annison, 1992). It has been proven that supplementation with exogenous xylanase is an effective solution to lower the viscosity of intestinal contents and improve digestibility of nutrients in broilers, leading to greater apparent metabolisable energy (AME) of wheat-based diets (Choct et al., 1992; Bedford and Morgan, 1996). It is known that the effectiveness of the enzyme addition is dependent on the source of xylanase and the quantity of wheat included in the diet. As a common field observation, and based on the summary of several research reports, the application of xylanase in wheat-based diets can improve broiler performance by 4-6% in terms of feed conversion ratio and increase the AME content of wheat by up to 6% (Cowieson et al., 2005, 2006; Scott, 2005). Marquardt et al. (1996) reported that wheat-based diets supplemented with exogenous xylanase could deliver identical, or even better, growth and feed conversion rate than unsupplemented corn-based diets.

The improvement in performance is closely associated with improvement in nutrient and energy utilization from the feed. Energy utilization in poultry is usually expressed in terms of AME which accounts for energy loss in the excreta. Most research on the effects of xylanase on energy utilization is based on the AME system, which does not consider the energy partition for production and heat production. It is expected that the net energy (NE) system will be more sensitive in measuring the response of broilers to xylanase application.

While the effects of xylanase on performance and nutrient utilization has already been well documented in

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broilers fed wheat based diet, there is a growing interest of studying the influence of exogenous enzyme addition on the microbial populations present in the digestive tract, due to the ban of antibiotics in several countries. Some authors have shown that the addition of xylanase can change the undigested nutrients entering in the hindgut and indirectly modify the microbial populations (Choct et al., 1999; Bedford, 2000; Cowieson et al., 2000), and as a consequence, the energy used for production in broilers was improved. The objective of the current study was to assess the effect of exogenous xylanase on the performance, net energy for production, nutrient utilization, and gut microflora of broilers fed wheat-based diet.

MATERIALS AND METHODS

Enzyme

A commercial xylanase product (Porzyme 9302; Danisco Animal Nutrition, Marlborough, UK) was used for this experiment. The xylanase was added in diets to provide a guaranteed minimum of 4000 U xylanase per kg feed, at an inclusion rate of 500 g/tonne. The definition of the xylanase unit is that 1 U is the amount of enzyme which liberates 0.5 μmol of reducing sugar (expressed as xylose equivalents) from a cross-linked oat spelt xylan substrate at pH 5.5 and 50°C in one minute.

Diets

The experimental design included 2 dietary treatments: i) a basal diet with reduced energy content, and ii) the basal diet supplemented with exogenous xylanase. The basal diet was formulated according to the commercial nutrient specifications used in China (NY/T33-2004), except that the AME was reduced by 150 kcal/kg to 2,850 kcal/kg in order to allow for a response to any additional energy provided by xylanase addition. The calculated dietary NE to AME ratio was 0.66. The NE levels of raw materials were adapted from de Groote (1974). In the second dietary treatment, the basal diet was supplemented with 500 g/t xylanase, providing a guaranteed minimum activity of 4,000 U/kg feed. Titanium oxide (4 g/kg) was added to both diets as an indigestible marker for digestibility measurements. The diets were fed in mash form. The basal diet formulation is shown in Table 1.

Table 1. Ingredient composition and calculated nutrient provision of the basal diet

Ingredients (kg/tone, as fed)	Basal diet
Wheat	751.42
Soybean meal	125.50
Corn gluten meal	72.69
Limestone	12.30
Dicalcium phosphate	16.50
Salt	3.60
L-lysine-HCl	6.66
DL-methionine	1.32
Threonine	1.71
Tryptophan	0.00
Vitamin premix*	0.20
Mineral premix**	2.00
Choline chloride	1.60
Zinc bacitracin	0.20
Antioxidant	0.20
Medical stone***	4.10
Calculated nutrient level	
Crude protein (%)	20.00
NE (kcal/kg)	1,895.00
AME (kcal/kg)	2,850.00
NE/AME	0.66
Calcium (%)	1.00
Total phosphorus (%)	0.68
Lysine (%)	1.15
Methionine (%)	0.50
Methionine and cysteine (%)	0.84
Tryptophan (%)	0.22
Threonine (%)	0.81
Total xylan (%)	6.05
Soluble xylan (%)	0.68

* Vitamin premix provided one kilogram of diet with: vitamin A, 10,800 IU; vitamin D_3, 2,160 IU; vitamin E, 151 IU; vitamin K_3, 1.0 mg; vitamin B_1, 4 mg; riboflavin, 5 mg; pantothenic acid, 10 mg; niacin, 25 mg; vitamin B_6, 8 mg; folic acid, 0.4 mg; vitamin B_{12}, 0.08 mg; biotin, 0.15 mg.

** Mineral premix provided one kilogram of diet with: I, 0.35 mg; Se, 0.15 mg; Zn, 40 mg; Cu, 8 mg; Fe, 80 mg; Mn, 100 mg.

*** Medical stone: a natural mineral additive, mainly contained of Al_2O_3 and SiO_2.

Birds and management

A total of 84, day-old male Arbor Acre chickens were purchased from a commercial hatchery (Beijing Arbor Acre Hatchery, Beijing, China). The chickens were allocated randomly to 2 dietary treatments, with 6 replicates per treatment and 7 chickens per replicate. All birds were housed in 3-deck, wire floored cages, and exposed to light for 24 h/d. The environmental temperature was maintained at 35°C during the first week, 33°C in the second week, 29°C in the third week and 25°C in the fourth week. Feed and water were offered *ad libitum* during the 30-day experimental period.

Sampling

On day 30, all birds were euthanized using the CO_2 for collecting digesta samples before killing. The small intestine was dissected and divided into four segments: duodenum, jejunum, ileum and caecum. Contents of the

distal part of the ileum from 4 birds per replicate were collected by flushing with distilled water. Digesta from birds within each replicate cage were pooled and frozen immediately. Subsequently digesta samples were freeze-dried and ground to pass though a 0.4-mm sieve (Mathlouthi and Lalles, 2002). Samples of diets, excreta and ileal digesta were assayed for nitrogen, energy, hemicellulose and titanium to estimate the digestibility of energy, nitrogen and hemicellulose.

The four small intestinal segments from 3 other birds from each replicate cage were frozen in liquid nitrogen and stored at -80°C until the determination of bacterial populations (Cao et al., 2003).

Measurements

Growth performance : Birds were weighed and feed intake recorded on days 1 and 29 on cage basis. Any bird that died during the experimental period was weighed and the weight recorded to adjust feed conversion ratio (FCR).

Respiratory chambers and heat production : This measurement was made using the auto-control, open circuit respiration calorimetry apparatus (KB-2, National Animal Nutrition Research Institute, China Agricultural University, China). The unit consisted of two chambers (A, B), a ventilation system, a temperature control system, a flow and gas analysis system (included an O×OR610 O_2 analyser, a QGS-08 CH_4 analyser and a QGS-08B CO_2 analyser), and a recorder. The dimension of each chamber was 380×240×200 cm (length×width×height). Each chamber can hold a single cage unit (170 cm length×80 cm width×190 cm height) consisting of an upper, a middle and a lower tier, each with two units in a back-to-back arrangement. Each unit contained 6 single-bird cages (85 cm length×80 cm width×95 cm height) fitted with individual galvanized iron feed troughs and small adjustable scoop channels. Plastic trays (90 cm length×80 cm width) were placed under each cage for the collection of excreta.

At 26 days of age, all replicates from each dietary treatment were placed in the chambers in order to adapt this environment. Chamber A contained the basal diet treatment, while chamber B contained the supplemented diet treatment. Excreta were collected at 8:00-10:00 am daily. The chambers remained sealed at all times, except during excreta collection. Initial reading of atmospheric pressure, temperature and humidity for each chamber were recorded. Room temperature was controlled by an air conditioning unit at 26±1°C to maintain a constant temperature within the chamber.

The respiratory quotient (RQ) during each run refers to the ratio of CO_2 produced by the birds to the volume of O_2 consumption (RQ = CO_2 produced/O_2 consumption). The value obtained indicates the degree of oxidation of diet on the trial. Heat production (HP) was calculated using the Brouwer equation (Brouwer, 1965) incorporated into the computer program. The measurement of HP was conducted over 3 days but was suspended for 2 h each day while the feed and water containers were replenished, excreta collected and the system readjusted for the next run. The HP was calculated on a 3-minute basis and then converted to a 24 h basis.

Apparent metabolisable energy and net energy : The AME determination was conducted from 26 to 29 day of age. AME was measured by recording feed intake and collecting all excreta per replicate cage for the entire 3-day period. Excreta were dried for 48 h at 65°C, then weighed and ground. The gross energy (GE) content of diets and dried excreta samples was determined. The AME value for each diet was calculated using AME = GE-EE (Excreta Energy). The NE_p value for each diet was calculated using NE_p = AME-HP.

Chemical analysis : Gross energy was determined using an oxygen bomb calorimeter (WZR-I A, Changsha, China), standardized with benzoic acid. Nitrogen content was determined by the Kjeldahl apparatus (KDY-9380, Shanghai, China). ADF and NDF content were measured following the method of van Soest et al. (1991), and hemicellulose content calculated. Titanium content was measured using a UV spectrophotometer following the method of Short et al. (1996).

Microbial populations in ileal and caecal contents : The contents from ileum and caecum (right section, 1 g) were homogenized with 99 ml of a sterile solution of 0.9% (w/v) NaCl and shaken vigorously for about 5 min. Further 10-fold serial dilutions were made. Lactobacilli were assessed on the LBS agar incubated aerobically and anaerobically (in an anaerobic cabinet) at 37°C for 48 h (GB/T 4789.35-2008, China). Bifidobacteria were cultured on BBL agar incubated anaerobically at 37°C for 48 h (GB/T 4789.34-2008, China). Coliform bacteria were measured on MacConkey agar incubated aerobically at 37°C for 24 h (GB/T 4789.38-2008, China). Salmonellas were cultured on S.S agar incubated aerobically at 37°C for 24 h (GB/T 4789.4-2008, China) .The counts were reported as $\log_{10}$ cfu/g of digesta.

Statistical analysis

All data were subjected to analysis of variance using the independent-sample *t*-test of SAS 8.2. Differences were considered significant at $p<0.05$.

RESULTS

Supplementation of xylanase had no effect ($p>0.05$) on the weight gain and feed intake of broilers (Table 2). But FCR was improved ($p<0.05$) by 4.3% with supplemental xylanase.

Table 2. Effect of xylanase on the growth performance of broilers fed wheat based diets

	Wheat (-xylanase)	Wheat (+xylanase)	Pooled SEM	p
Body weight gain (g/bird)	827.8	858.7	16.23	0.365
Feed intake (g/bird)	1,437.5	1,452.2	18.68	0.714
FCR (g/g)	1.772[a]	1.696[b]	0.020	0.048

Table 3. Effect of xylanase on the energy utilization by birds fed wheat based diets

	Wheat (-xylanase)	Wheat (+xylanase)	Pooled SEM	p
AME (MJ/kg DM)	11.93[b]	12.43[a]	0.12	0.032
HP (MJ/kg DM)	5.55[a]	4.40[b]	0.28	0.006
Hp (MJ/d/kg$^{0.75}$)	0.622[a]	0.459[b]	0.039	0.004
NE_p (MJ/kg DM)	6.37[b]	8.03[a]	0.27	0.000
V_{CO2} (L/d/kg$^{0.75}$)	29.54[a]	22.93[b]	1.60	0.009
V_{O2} (L/d/kg$^{0.75}$)	28.97[a]	21.25[b]	1.80	0.003
RQ	1.025[b]	1.087[a]	0.15	0.009

The addition of xylanase increased ($p<0.05$) diet AME by 0.5 MJ/kg DM while heat production was reduced ($p<0.05$) by 1.15 MJ/kg DM, consequently improving ($p<0.05$) net energy for production by 1.7 MJ/kg DM. CO_2 production and O_2 consumption were reduced ($p<0.05$), RQ was increased ($p<0.05$) by xylanase addition to the wheat-based diet (Table 3). Ileal digestibility of nitrogen and energy was improved by 3 and 6 percentage units, respectively, but the differences were not statistically significant ($p>0.05$, Table 4). Ileal digestibility of hemicellulose was significantly ($p = 0.05$) improved by xylanase supplementation.

Xylanase supplementation increased ($p<0.05$) the counts of coliform, salmonella, lactobacillus and bifidobacterial in the caecal content (Table 5). In the ileal contents, the counts of coliform, bifidobacterial were reduced ($p<0.05$) and salmonella was not detected when the birds were fed the diet with xylanase. Lactobacillus count was increased ($p<0.05$) with added xylanase.

DISCUSSION

Arabinoxylans are the main non starch polysaccharides (NSP) in wheat that increase viscosity of digestive content in the small intestine and interfere with digestion and absorption of nutrients when fed to broilers. As a result, feed conversion efficiency and growth are reduced and the incidence of pasting vents is increased (Friesen et al., 1992; Marquardt et al., 1994). Xylanase supplementation can break down plant cell wall, reduce their integrity and thus release nutrients that were previously encapsulated to improve digestive function and to promote animal performance (Bedford, 2000). As expected, the present study demonstrated the positive effect of xylanase

Table 4. Effect of xylanase on the ileal nutrient and energy digestibilities in broilers fed wheat-based diets

	Wheat (-xylanase)	Wheat (+xylanase)	Pooled SEM	p
N digestibility	80.09	83.08	1.53	0.358
GE digestibility	61.31	67.49	2.02	0.133
Hemicellulose digestibility	28.41[b]	48.63[a]	5.05	0.050

Table 5. Effect of xylanase on the counts of bacteria in the ileum and caecum (log_{10} cfu/g) of broilers fed wheat-based diets

	Caecum				Ileum			
	Wheat (-xylanase)	Wheat (+xylanase)	Pooled SEM	p	Wheat (-xylanase)	Wheat (+xylanase)	Pooled SEM	p
Coliforms	5.98[b]	7.63[a]	0.32	0.004	6.82[a]	4.57[b]	0.45	0.002
Salmonella	3.86[b]	5.56[a]	0.40	0.033	5.59[a]	0.00[b]	1.12	0.000
Lactobacillus	10.23[b]	10.92[a]	0.17	0.043	5.65[b]	6.90[a]	0.25	0.009
Bifidobacteria	10.24[b]	11.33[a]	0.21	0.004	11.50[a]	9.76[b]	0.41	0.038

supplementation in wheat-based diets for broiler chickens. Feed conversion ratio was improved by xylanase addition, which is in agreement with earlier reports (Adeola and Bedford, 2005; Gao et al., 2008). Friesen et al. (1992) showed that the improvement in terms of bodyweight gain and feed conversion of broiler chickens fed with diets containing 70% of wheat to be 2.9% and 9.2%, respectively. Wang et al. (2003) reported that the addition of xylanase to the wheat-based diet improved the performance of broilers and even a better performance was achieved than those fed corn-based diets.

The improvement in feed conversion ratio observed in this study is likely to be a result of the improved nutrient and energy utilization. Present data showed that xylanase supplementation increased the AME by 0.5 MJ/kg DM. This was probably a consequence of the increase in hemicellulose digestibility. By breaking down arabinoxylan polymers from wheat grain, xylanase has been shown to reduce gut viscosity and nutrient entrapment (mostly starch and protein), and lead to better digestion in broilers (Bedford, 2000).

The key finding of this study is that the addition of xylanase has increased the net energy for production and reduced the energy for heat production. The likely interpretation for such reduction is that the dietary fibre often contribute significant amount of heat production after ingestion. Xylanase addition can effectively break down the dietary fibre and lead to reduction in the weight and relative proportion of energetically active organs like the gastro-intestinal tract and pancreas in pigs (Shelton et al., 2003; Kies et al., 2005) and poultry (Esteve-Garcia et al., 1997; Wu et al., 2004), consequently reduce the heat increment and the total cost of maintenance. Gao et al. (2008) showed that the addition of xylanase to a wheat-based diet significantly reduced the relative weights of the duodenum, jejunum, colon and pancreas in 21-day-old broiler chickens. Furthermore, the addition of xylanase can reduce intestinal viscosity, decrease the secretion of endogenous protein, water, minerals and fatty acid, and consequently improve energy efficiency (Wang, 2003). The reduction in heat production, and the consequent improvement in energy for production (NEp) observed in the current study with xylanase addition not only support the improvements in the FCR, but also indicatest that net energy system may be more sensitive for assessing enzyme effect on energy utilisation.

Energy evaluation in poultry is historically based on the apparent metabolisable energy (AME) system, but the system is not capable of accounting for losses of chemical energy in the solid, liquid and gaseous excreta or as heat (Pirgozliev and Rose, 1999). For many decades, it has been debated whether or not the development of a net energy system would enhance the prediction of energy partitioning in poultry. It has been believed that gaseous energy losses from the digestive tract are too small to affect the estimate of ME significantly, but the results from current study demonstrated that net energy is significantly associated with xylanase responses while the AME is not a good indicator of the enzyme value when it is used for feed formulation. Daskiran et al. (2004) using the carbon-nitrogen method showed that a carbohydrase improved NE in a maize-soy bean meal without any change in ME.

Some experiments have shown that xylanase addition could inhibit the proliferation of potentially pathogenic microbial populations in the intestine, improve animal health. The reduction in viscosity by xylanase can speed up the rate of stomach emptying, reduce intestinal fermentation, inhibit the growth of anaerobic microorganisms and reduce the incidence of intestinal diseases. Schutte et al. (1995) reported that xylanase reduced the number of bacteria in the gastrointestinal tract in wheat or rye diet. Xylanase was found to significantly reduce the intestinal bacteria and total anaerobic bacteria (Dänicke et al., 1999). Vahjen (1998) reported that xylanase could reduce the adhesion of bacteria in the intestine when added to wheat-based diets for broilers. In the current study, adding xylanase in the diet reduced the number of coliform and salmonella, and increased the number of lactobacillus in the ileum. This is consistent with other findings (Choct et al., 1999). However, the increase in the number of coliforms and salmonella in the caecal contents is difficult to explain. It is known that enzyme could increase the fermentation in the caecum, which presumably was due to the lower molecular weight carbohydrates entering the caecum (J´ozefiak et al., 2004). Choct et al. (1999) reported that xylanase supplementation in wheat-based diet decreased the intestinal digesta viscosity and volatile fatty acids in the ileum of broilers, while the caecal volatile fatty acids increased significantly, presumably due to the increased fermentation by bacteria. Gao et al. (2008) reported that xylanase addition in wheat-based diets had no significant effects on the bifidobacterium, lactobacillus and *E. coli* in the ileum and caecum of broilers. However, it should be pointed out that the increase in the number of bacteria in caecum did not cause any negative effect on the performance of broilers in the current study.

CONCLUSIONS

The addition of xylanase to a wheat based diet significantly reduced heat production, leading to greater NE and a better FCR of broilers. Net energy is a more sensitive and/or accurate parameter for assessing the value of xylanase for poultry. The application of xylanase could reduce the number of coliform and salmonellas in ileum

which is crucial for bird health and food safety.

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REFERENCES

Adeolal, O. and M. R. Bedford. 2005. Exogenous dietary xylanase ameliorates viscosity-induced anti-nutritional effects in wheat-based diets for White Pekin ducks (Anas platyrhynchos). Br. J. Nutr. 92:87-94.

Bedford, M. R. and A. J. Morgan. 1996. The use of enzymes in poultry diets. World Poult. Sci. J. 52:61-68.

Bedford, M. R. 2000. Exogenous enzymes in monogastric nutrition-their current value and future benefits. Anim. Feed Sci. Technol. 86:1-13.

Brouwer, E. 1965. Report of sub-committee on constants and factors. In: 3rd Symposium on Energy Metabolism Troon (Ed. K. L. Blaxter). European Association of animal production publication. Academic Press. London. pp. 441-443.

Cao Binghai, Zhang Xiuping, Guo Yuming, Yuan Jianmin and Nie Wei. 2003. Effect of TP and FOS supplementation on performance and caecal microflora counts and its metabolites in female broiler chickens fed semi-purified diets. J. China Agric. Univ. 8(3):85-90.

Choct, M. and G. Annison. 1992. The inhibition of nutrient digestion by wheat pentosans. Br. J. Nutr. 67:123-132.

Choct, M., G. Annison and R. P. Trimble. 1992. Soluble wheat pentosans exhibit different anti-nutritive activities in intact and cecectomised broiler chickens. J. Nutr. 122:2457-2465.

Choct, M., R. J. Hughes and M. R. Bedford. 1999. Effects of xylanase on individual bird variation, starch digestion throughout the intestine, and ileal and caecal volatile fatty acid production in chickens fed wheat. Br. Poult. Sci. 40:419-422.

Cowieson, A. J., L. K. Sarkilahti, J. H. A. Apajalahti, T. Acamovic and M. R. Bedford. 2000. Caecal microflora in broilers fed diets containing camelina with and without enzyme supplementation. In Proceedings of the XXI World's Poultry Congress.CD ROM. Montreal, Quebec, Canada: World's Poultry Science Association. Candian Branch.

Cowieson, A. J., M. Hruby, Faurschou and M. Isaksen. 2005. The effect of conditioning temperature and exogenous xylanase addition on the viscosity of wheat-based diets and the performance of broiler chicks. Br. Poult. Sci. 46:717-724.

Cowieson, A. J., M. Hruby and E. E. M. Pierson. 2006. Evolving enzyme technology: impact on commercial poultry nutrition. Nutr. Res. Rev. 19:90-103.

Dänicke, S., W. Vahjen, O. Simon and H. Jeroch. 1999. Effects of dietary fat type and xylanase supplementation to rye-based broiler diets on selected bacterial groups adhering to the intestinal epithelium, on transit time of feed, and on nutrient digestibility. Poult. Sci. 78(9):1292-1299.

Daskiran, M., R. G. Teeter, D. Fodge and H. Y. Hsiao. 2004. An evaluation of endo-beta-d-mannanase (Hemicell) effects on broiler performance and energy use in diets varying in beta-mannan content. Poult. Sci. 83:662-668.

Esteve-Garcia, E., J. Brufau, A. Perez-Vendrell, A. Miquel and K. Duven. 1997. Bioefficacy of enzyme preparations containing beta-glucanase and xylanase activities in broiler diets based on barley or wheat, in combination with flavomycin. Poult. Sci. 76:1728-1737

Gao, F., Y. Jiang, G. H. Zhou and Z. K. Han. 2008. The effects of xylanase supplementation on performance, characteristics of the gastrointestinal tract, blood parameters and gut microflora in broilers fed on wheat-based diets. Anim. Feed Sci. Technol. 142:173-184.

Friesen, O. D., W. Guenter,R. R. Marquardt and B. A. Rotter. 1992. The effect of enzyme supplementation on the apparent metabolizable energy and nutrient digestibilities of wheat, barley, oats and rye for young broiler chicks. Poult. Sci. 71: 1710-1721.

De Groote, G. 1974. A comparson of a new net energy system with the metabolisable energy system in broiler diet formulation, performance and profitability. Br. Poult. Sci.15:75-95.

J´ozefiak, D., A. Rutkowski and S. A. Martin. 2004. Carbohydrate fermentation in the avian ceca-a review. Anim. Feed Sci. Technol. 113:1-15.

Kies, A. K., W. J. S. Gerrits, J. W. Schrama, M. J. W. Heetkamp and K. L. Van der Linden. 2005. Mineral absorption and excretion as affected by microbial phytase, and their effect on energy metabolism in young piglets. J. Nutr. 135:1131-1138.

Marquardt, R. R., D. Boros, W. Guenter and G. Crow. 1994. The nutritive value of barley, rye, wheat and corn for young chicks as affected by use of a *Trichoderma reevei* enzyme preparation. Anim. Feed Sci. Technol. 45:363-378.

Marquardt, R. R., A. Brenes and Z. Zhang. 1996. Use of enzymes to improve nutrient availability in poultry feedstuffs. Anim. Feed Sci. Technol. 60(3):321-330.

Mathlouthi, N. and J. P. Lalles. 2002. Xylanase and β-glucanase supplementataion improve conjugated bile acid fraction in intestinal contents and increase villus size of small intestine wall in broiler chickens fed a rye-based diet. Anim. Sci. 80:2773-2779.

Pirgozliev, V. and S. P. Rose. 1999. Net energy systems for poultry feeds: a quantitative review. World Poult. Sci. J. 55:23-36.

SAS Institute. 2005. SAS/STAT User's Guide. Release 8.2.Cary, NC: SAS Institute.

Schutte, J. B., L. De Jong and D. J. Langhout. 1995. Effect of an endoxylanase preparation in wheat based rations for broilers. Feed Compounder. 15(1):20-24.

Scott, T. A. 2005. The impact of pelleting and enzyme supplementation on feed value of twenty-five Canadian wheat samples. Proceedings of the Australian Poultry Science Symposium 17:138-144.

Shelton, J. L., L. L. Southern, T. D. Bidner, M. A. Persica, J. Braun, B. Cousins and F. McKnight. 2003. Effect of microbial phytase on energy availability, and lipid and protein deposition in growing swine. J. Anim. Sci. 81:2053-2062.

Short, F. J., P. Gorton and J. Wiseman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59:215-221.

Vahjen, W., K. Gläser and K. Schäfer. 1998. Influence of xylanase-supplemented feed on the development of selected bacterial groups in the intestinal tract of broiler chicks. J. Agric. Sci. 130(4):489-500.

Van Soest, P. J., J. B. Robertson and B. A. Lewis. 1991. Symposium: Carbohudrate Methodology, Metabolism, and Nutritional Implications in Dairy Cattle. Methods for dietary fibre, neutral detergent fibre, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Wang, H. Y., Y. M. Guo and J. M. Yuan. 2003.The effect of xylanase on performance in broilers fed on wheat diets. Feed Study 12:1-5.

Wu, Y. B., V. Ravindran, D. G. Thomas, M. J. Birtles and W. H. Hendriks. 2004. Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolisable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. Br. Poult. Sci. 45:76-84.