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Dietary Pentosanase Supplementation of Diets Containing Different Qualities of Wheat on Growth Performance and Metabolizable Energy of Turkey Poults

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Abstract: Wheat varies in apparent metabolizable energy N-corrected (AMEn) due to the presence of non-starch polysaccharides (NSP), which can be improved by dietary enzyme supplementation. Poults from 0-17 d-age were fed diets containing various wheat sources (WS) with or without Natugrain Blend® (NB) (BASF, Germany). Five replicate cages of 10 poults were assigned to each eight-soybean-meal/wheat treatment diets and a control soybean-meal/corn diet. The treatments were a factorial arrangement of 4 WS (A, B, C, D) and 2 enzyme levels (0 and 200 mg NB/kg). The WS differed by the degree of frost damage during seed development. Regardless of the source of wheat, NB increased 17 d BW (351 vs 381 g, $P < 0.001$), decreased 1-17 d FCR (1.55 vs 1.49, $P < 0.05$), increased AMEn (2,204 vs 2,455 kcal/kg, $P < 0.001$), and increased apparent nitrogen retention (ANR) (35.0 vs 41.4 %, $P < 0.05$). No effects of WS were seen on growth performance, but WS A and B had higher ($P < 0.05$) AMEn than sources C and D (2,396 and 2,460 vs 2,246 and 2,216 kcal/kg, respectively). Gut viscosity was higher ($P < 0.05$) in poults fed wheat-based diets than the control diet. Enzyme supplementation to the wheat-based diets decreased viscosity (5.57 vs 3.98 cP, $P < 0.05$) to a level similar to the corn-based control diet, and it resulted in equivalent growth performance. Viscosities were negatively correlated with AMEn. The results demonstrated a positive effect of enzyme supplementation on nutrient utilization and performance of turkeys.

Key words: Wheat, enzymes, growth performance, metabolizable energy, turkey

Introduction

Wheat is an important feed ingredient for poultry in many parts of the world. Although it can supply up to 70% of the metabolizable energy in the feed, the dietary inclusion rate of wheat is often limited because of the variability of apparent metabolizable energy (AME). Wheat may contribute up to 35% of the protein and 25% of the lysine in a broiler diet when included at high concentrations. However, the greatest nutritional significance comes from starch, which is the major energy-yielding component (Wiseman *et al*., 2000).

Water-soluble β-glucans and arabinoxylans are the non-starch polysaccharides (NSP) of major concern when being fed in poultry diets with high cereal grain content. β-Glucans are linear polymers of glucose with β-(1,3)(1,4) glycosidic links (Fincher and Stone, 1986). Arabinoxylans consist of long backbone chains of β-(1,4) anhydro-D-xylopyranosyl to which are attached single α-L-arabinofuranosyl residues at the 2- or 3-position. The predominant NSP in wheat are mainly arabinoxylan and some β-glucans. Researchers have reported that the occurrence of low AME of wheat is caused by the presence of soluble NSP in cell walls of the wheat kernels (Mollah *et al*., 1983; Annison, 1993). Water-soluble pentosans of wheat have the ability to bind large quantities of water (Bushuk, 1966), and to form viscous gels in the digesta of poultry *via* covalent cross-linking (Geissmann and Neukom, 1973). The viscous gel lowers the rate of diffusion of nutrients in the digesta and it acts as a physical barrier that impedes the interactions between substrates, enzymes, and digestion end-products (Pettersson and Aman, 1989). Consequently, NSP compromises enteric digestion and nutrient absorption. By increasing digesta viscosity, NSP reduces digesta mixing, feed passage rate, and luminal oxygenation; thus causing an increase in the proliferation of microflora and fermentation in the small intestine (Preston *et al*., 2001). Some enteric microflora compete with the host for nutrients, reduce fat absorption by deconjugating bile salts (Coates *et al*., 1981; Feigner and Dashkevicz, 1988), and cause morphological changes in the gut villi by irritating the gut lining and damaging microvilli (Visek, 1978). Thus, some microflora adversely affects digestive processes by limiting the digestibility and absorption of nutrients.

Dietary pentosanase (e.g. arabinoxylanase, β-glucanase) supplementation is commonly used as a mean to improve the feeding value of wheat for poultry. Appropriate enzyme supplementation to wheat-based diets improves the digestion of dietary starch, protein, and lipid in the small intestine, and it results in more consistent and uniform poultry performance (Choct *et al*., 1999). Supplementing cereal-based diets with microbial enzyme preparations capable of hydrolyzing endosperm cell walls may improve dietary nutrient availability by degrading the xylan backbone of arabinoxylan into smaller units that reduces the viscosity of the digesta in the small intestine (Bedford and Schulze, 1998; Choct *et*

al., 1999). Reduced digesta viscosity increases the diffusion rates of nutrients and endogenous enzymes and decreases the proliferation of the microflora enabling the bird to digest and absorb more nutrients (Pawlik *et al.*, 1990; Choct *et al.*, 1999). The beneficial effects of enzymes are more apparent if diets are supplemented with a blend of enzymes (enzyme cocktail) than single-activity enzymes (Bedford and Classen, 1992a; Odetallah *et al.*, 2002).

The effects of enzyme supplementation in wheat-based diets are dependant upon the source and type of wheat involved. Veldman and Vahl (1994) studied the effect of four wheat varieties supplemented with xylanase on the growth performance of broilers. The enzyme had a different effect on broiler performance for each variety of wheat. Saulnier *et al.* (1995) attribute variations in enzyme effects among wheat cultivars to the high heterogeneity in water-soluble arabinoxylan content. Wootton *et al.* (1995) reported that the pentosan content ranged from 5.4 to 7.2% in Australian wheat and from 5.5 to 6.5% in North American wheat samples harvested in 1992 to 1993. Choct *et al.* (1999) reported that the AME values of the Australian wheat differ significantly due to year of harvest, with the values ranging from 2,194 kcal/kg to 3,580 kcal/kg (dry matter basis - DM). They suggested that the AME values of the wheat were dependent upon the climatic condition during the growing season: i.e. wet conditions during grain maturation dramatically decrease the extract viscosity of the grain, whereas dry conditions during grain maturation elevate its viscosity. Aastrup (1979) observed a similar response in barley. Wheat also shows higher levels of pentosans when frost-damaged during seed maturation (immature wheat) (Ward, 1995). However, the effect of enzyme(s) on immature frost-damaged wheat fed to turkeys has not been reported. Therefore, the NSP level varies widely depending on environmental factors and variety.

The purpose of this research was to compare the nutritional value of immature frost-damaged wheat with mature wheat for turkeys, and how a blend of NSP degrading enzymes influences the nutritive value of such wheat.

Materials and Methods

Enzymes: The enzyme used in this study was Natugrain Blend®[1] (NB), a liquid enzyme preparation obtained from fungal fermentation of *Trichoderma longibrachiatum*. It contains standardized activities of β-glucanase and endoxylanase of at least 9,000 β-glucanase units (BGU) per gram of product and at least 36,600 endoxylanase units (EXU) per gram of product (G. K. Holkenborg, 2001, BASF, Aktiengesellschaft, Ludwigshafen, Germany, personal communication). One BGU is defined as the activity required to liberate 0.278 µmol of reducing sugar (measured as glucose equivalents) per minute at pH 3.5 and 40 °C at a substrate concentration of 0.5% β-glucan from barley. One EXU is defined as the enzyme activity required to liberate 1 µmol of reducing sugar (measured as glucose equivalents) per minute from a 1% xylan solution at pH 3.5 and 40 °C. Natugrain Blend® also contains some hemicellulase (e.g. pectinase), cellulase and protease activities (BASF, 1997). The enzyme dosage was 200 g NB/tonne of feed, as recommended by the manufacture. Thus, the enzyme concentration in the feed was at least 7,300 EXU/kg and 1,800 BGU/kg.

Diets: The experimental diets are presented in Table 1. The chemical composition of the wheat used in the experimental diets is shown on Table 2. A basal diet containing all ingredients except corn or wheat was prepared as a single batch. Then the basal diet was split into 9 equal portions. One portion was used for the corn-based diet and 8 for the wheat diets. The 8 wheat-based dietary treatments were a factorial arrangement of 4 wheat sources (A, B, C, and D) and 2 enzyme supplementation levels (0 and 200 mg NB/kg).

All feeds were formulated using least-cost linear programming software, such that the diets were about 95% of the NRC (1994) recommendations for amino acids and energy. The diets were formulated slightly below requirements so that any improvement in nutrient availability due to enzyme supplementation could be observed as an improvement in growth performance. The corn-based control diet was prepared by mixing 50% corn, 0.1% Solka-Floc[2] (cellulose), and 49.9% basal diet. The wheat-based diets were prepared by mixing 50% wheat (sources A, B, C, or D), 0.1% Solka-Floc or enzyme, and 49.9% basal diet. Half of the wheat diets were supplemented with NB (200 mg/kg diet) and the other half of the wheat diet had no NB supplementation. The enzyme was applied as a fine spray onto the feed using a plant mister[3]. Celite[4], a source of acid-insoluble ash, was used as an indigestible marker at 0.7% of the total diet. All feeds were prepared and fed as a mash. Composite feed samples from each diet were taken immediately after manufacture and analyzed for crude protein, fat, ash, Ca, and P.

Wheat: The wheat sources differed by the degree of frost damage during seed development. The wheat was all of the same variety grown on the same farm in Saskatchewan, Canada. The 1999 wheat-growing season in Saskatchewan, which usually starts in the second half of April, was delayed by heavier than normal snow cover until the first week of May and excessive soil moisture levels due to high precipitation until the first half of June (Morgan, 1999). Temperatures in May, June and July also were cooler than normal, with reporting deviations of 0.5 °C to 3 °C below normal. This cooler condition prolonged crop development. In August of

Table 1: Composition and calculated nutrient content of the experimental diets fed to turkeys from 1 to 17 days of age

Ingredient	Basal diets	
	Corn	Wheat
	(%)	
Corn	50.0	0
Wheat A, B, C, or D[1]	0	50.0
Soybean meal (48%)	39.0	39.0
Poultry meal (60%)	5.0	5.0
Dical (18.5% P)	2.05	2.05
Calcium Carbonate	1.44	1.44
Salt	0.29	0.29
DL-Methionine	0.19	0.19
L-Lysine Hcl	0.15	0.15
Minerals[2]	0.20	0.20
Vitamins[3]	0.10	0.10
Choline Cl (60%)	0.20	0.20
Selenium Premix[4]	0.075	0.075
Celite[5]	0.70	0.70
Enzyme or Solka-Floc[6]	0.10	0.10
Titanium Oxide	0.50	0.50
Calculated Analysis		
ME, Kcal/kg	2,766	2,676
Crude Protein, %	26.7	27.4
Methionine + Cystine, %	1.05	1.02
L-Lysine, %	1.60	1.60
Calcium, %	1.25	1.27
Non-Phytate Phosphorus, %	0.60	0.64
Sodium, %	0.17	0.18

[1]The wheat sources differed by the degree of frost damage during seed development. Wheat A and B were frost damaged near full maturity, and wheat C and D were damaged during grain filling stage (mid-milk to soft-dough stage). [2]Supplied the following per kilogram of feed: 120 mg Zn as $ZnSO_4.H_2O$; 120 mg MN as $MnSO_4.H_2O$; 80 mg Fe as $FeSO_4H_2O$; 10 mg Cu as $CuSO_4$; 2.5 mg I as $Ca(IO_3)_2$; 1.0 mg Co as $CoSO_4$. [3]Supplied the following per kilogram of feed: vitamin A, 13,200 IU; cholecalciferol, 4,000 IU; niacin, 110 mg; pantothenic acid, 22 mg; riboflavin, 13.2 mg; pyridoxine, 7.9 mg; menadione, 4 mg; folic acid, 2.2 mg; thiamin, 4 mg; biotin, 0.253 mg; vitamin B_{12}, 0.04 mg; ethoxyquin, 100 mg. The vitamin E premix provided the necessary amount of vitamin E as DL-α-tocopheryl acetate. [4]Selenium premix supplied 0.3 ppm Se as sodium selenate. [5]Celite (CeliteTM, Celite Corp., Lompar, CA 93436), a source of acid-insoluble ash, were used as an indigestible marker. [6]Enzyme treatments were supplemented with enzyme products that accounted as a dry ingredient (0.1%) and an equivalent amount of Solka-Floc (cellulose) was applied to the unsupplemented treatments.

1999 in the Saskatchewan region, warm temperatures and a decrease in rain helped the crop to develop, although most plantings were 10 to 15 days behind normal development (Morgan, 1999). Wheat A, B, C, and D utilized in this research were planted in the second, third, and fourth week of May and first week of June, respectively. In Saskatchewan, the harvest period usually starts early September and finishes at the end of October (Morgan, 1999). In the Saskatchewan region temperatures usually average 5 to 17 °C in September that helps the crop to reach maturation before harvest. In the case of the wheat samples tested, an early fall frost event occurred in the first week of September when wheat samples A and B were near full maturity (mid-to end of ripening), and when wheat C and D were in the milk to the soft-dough stage of seed development (mid-milk to the mid-dough stage). The four wheat groups were harvested within the same week during the fall of 1999. After harvest, all wheat sources were stored for about 8 months before being used in the experiment.

The milk stage is defined as when the color and consistency of the kernel resembles that of milk. The endosperm thickens as the grain progresses into the dough stage. The dough stage is often further sub-divided into soft and hard dough stages, as the moisture content of the grain decreases and it becomes more difficult to split with a fingernail. The wheat reaches maturity as the grain hardens further and loses moisture to 30 to 35%. It typically takes 30 days for wheat to reach physiological maturity from flowering (Simmons *et al.*, 1995), and a total of 103 days from planting (Fowler and Hermenean, 1996). However, variation in this growth period occurs because temperature determines the rate of wheat development.

Bird Husbandry and Nutrient Digestibility (AMEn and ANR) Bioassay: One-day-old commercial Large White BUTA[5] male poults were obtained from a commercial hatchery[6] and were randomly assigned to cages. Wing bands[7] were applied to the birds at the time of placement to identify their treatment assignment. All birds were housed in four Petersime battery cage brooder units[8] within a climate-controlled animal room. Each 12-cage battery unit was considered as an experimental block to account for error due to differences in temperature, light, or position. All cages contained individual heating units that were monitored throughout the trial for consistency. The brooder unit's temperature was set at 35 °C and was then altered as needed to suit bird comfort. Room temperature was set at 28 °C on the day of placement, and was subsequently reduced 2 °C per week. The room was illuminated with incandescent lights at about 50 lux on a continuous basis. Feed and water were provided *ad libitum* throughout the duration of the study. Visual heath inspection of all birds within the study was performed daily and weights of culled

Table 2: Chemical composition of wheat used in the experimental diets fed to turkeys from 1 to 17 days of age

Analysis	Wheat Type[1]			
	A	B	C	D
Gross Energy, Kcal/kg	4,240	4,430	4,430	4,430
Crude Protein, %	13.7	16.0	15.9	16.5
Crude Fat, %	1.50	1.60	1.70	1.70
L-Lysine, %	0.34	0.38	0.38	0.40
Fiber (ADF), %	3.30	3.70	3.80	4.20
Fiber (NDF), %	19.8	17.9	20.7	20.6
Ash, %	2.17	1.93	2.10	2.20
Calcium, %	0.05	0.05	0.05	0.06
Phosphorus, %	0.49	0.47	0.33	0.40
Sodium, %	0.02	0.02	0.02	0.02

[1]The wheat sources differed by the degree of frost damage during seed development. Wheat A and B were frost damaged near full maturity, and wheat C and D were damaged during grain filling stage (mid-milk to soft-dough stage).

birds and reasons they were removed were recorded. Crippled or dead birds were removed and replaced up through day three, at which time any further mortality was removed and recorded but not replaced. All mortalities were weighed soon after death and recorded so that their weights could be included in the calculation of feed conversion.

Nine dietary treatments were randomly assigned to cages within each of the four blocks using the Proc-Plan procedure of SAS® (SAS, 1996). Each cage of 10 poults, the experimental unit, were subjected to one of nine dietary treatments from 1 to 17 days of age, as follow: (1) corn/soybean meal unsupplemented control, (2) wheat A no enzyme, (3) wheat A with enzyme, (4) wheat B no enzyme, (5) wheat B with enzyme, (6) wheat C no enzyme, (7) wheat C with enzyme, (8) wheat D no enzyme, and (9) wheat D with enzyme. Five replicate cages were used for each treatment combination.

Data Collection: Group feed consumption (g) and body weights (g) were recorded at 1, 7, 13, and 17 days of age. Fecal collections were done daily from 9:00 to 11:00 am on days 11 to 14. The samples were put into plastic bags[9] and stored frozen at minus 20 °C until chemical analysis associated with the determination of apparent metabolizable energy nitrogen-corrected (AMEn) and apparent nitrogen retention (ANR).

Dietary Viscosity Measurement: At 17 days of age, the birds were fasted over night (8 hours) and then given *ad libitum* access to feed for 3 hours before sampling. Two birds from each cage were weighed and euthanized by cervical dislocation. About 2 grams of digesta was then gently expressed from the terminal part of the jejunum (midway between the duodenum and Meckel's diverticulum to 0.5 cm above the Meckel's diverticulum), and was then placed into micro-centrifuge tubes, centrifuged[10] at 3,000 rpm for 2 minutes, and the supernatant was collected. The viscosity of the supernatant was determined using Brookfield Digital Viscometer LVDVII+CP[11].

Chemical Analysis: The frozen fecal samples, that had been collected daily from days 11 to 14, were thawed overnight at room temperature and were then pooled (approximately total of 300 g excreta) and mixed in a blender[12] to slurry after the addition of approximately 100 ml distilled water. The pH of the fecal slurry was then adjusted to 5.4 by the addition of sulfuric acid (0.1 N) to minimize the volatilization of nitrogen during overnight drying at 70 °C in a forced-air convection oven[13]. The dried samples were ground in a blender[12], and were then stored at minus 20 °C before analysis to determine AMEn and ANR. Dry matter content was obtained by drying 3 to 5 grams of the sample material for 6 hours in a forced-air convection oven[13] at 105 °C. Feed and fecal energy values were obtained by combustion in an adiabatic oxygen bomb calorimeter[14]. Celite recovery was performed using the method described by Vogtmann *et al.* (1975). The nitrogen content of all samples was determined using a Kjeldahl automatic nitrogen analyzer[15]. The AMEn and ANR values were calculated relative to the acid-insoluble ash marker as shown in the footnote of Table 6. Correction of the AMEn to zero N-retention was accomplished by using a value of 8.22 kcal/g nitrogen retained (Hill and Anderson, 1958).

Statistical Analysis: All data were analyzed using the general linear models procedure for ANOVA (SAS, 1996). Cage means served as the experimental units for statistical analysis. Variables having a significant F-test were compared using the least-squares-means function of SAS® (SAS, 1996), and the treatment effects were considered to be significant at $P < 0.05$. All percentage data were transformed to arc sine of the square root to

Table 3: Effects of wheat source[1] with (+) and without (-) enzyme (Natugrain Blend®[2]) supplementation on body weight of poults raised from 0 to 17 days of age

Dietary Treatments		Days of Age		
Cereal Base	Enzyme	7	13	17
		(g)[3]		
Corn	-	143.3[a]	248.7[d]	353.7[bcd]
Wheat A	-	129.8[c]	253.7[cd]	364.3[abcd]
Wheat A	+	139.9[ab]	271.7[ab]	376.4[abc]
Wheat B	-	128.3[c]	257.1[bcd]	349.4[bcd]
Wheat B	+	142.6[a]	272.7[ab]	380.5[ab]
Wheat C	-	132.9[bc]	255.5[cd]	348.0[cd]
Wheat C	+	135.3[abc]	265.7[bc]	370.9[abcd]
Wheat D	-	131.7[bc]	248.1[d]	341.1[d]
Wheat D	+	144.8[a]	283.6[a]	394.2[a]
P-Value		0.0043	0.0005	0.0227
SEM(36)[4]		3.35	5.57	10.89
Main effect of enzyme supplementation among wheat treatments		(g)[5]		
Enzyme -		130.7[b]	253.6[b]	350.7[b]
Enzyme +		140.7[a]	273.4[a]	380.5[a]
Source of variation among wheat treatments		(P-Value)		
Enzyme		0.0002	0.0001	0.0007
Wheat		0.6360	0.7840	0.7957
Wheat X enzyme		0.2955	0.1471	0.3272
SEM(32)[6]		3.37	5.60	11.25

[a-d]Means with different superscripts within a column differ significantly ($P < 0.05$). There were no significant differences in poults starting weights at 1 d of age (60g). [1]The wheat sources differed by the degree of frost damage during seed development. Wheat A and B were frost damaged near full maturity, and wheat C and D were damaged during grain filling stage (mid-milk to soft-dough stage). [2]Natugrain Blend® contains 36,600 EXU/g of xylanase activity; 9,000 BGU/g of β-glucanase activity; and some hemicellulose, cellulase and protease activities (BASF, 1997). The dosage used was 200 mg/kg of feed, as recommended by the manufacture.
[3]Values represent means of 5 cages containing 10 poults each. Data from 9 treatments (1 corn, 8 wheat).
[4]SEM(36) = Standard Error of the mean with 36 degrees of freedom. [5]Values represent means of 20 cages containing 10 poults each. Data from 8 wheat treatments (4 wheat sources and 2 level enzyme). [6]SEM(32) = Standard Error of the mean with 32 degrees of freedom.

the data distribution before statistical analysis. Correlation analysis of digesta viscosity and AMEn and ANR were performed using Proc Corr procedure (SAS, 1996).

Animal Ethics: The experiments reported herein were conducted according to the guidelines of the Institutional Animal Care and Use Committee at North Carolina State University. All husbandry practices and euthanasia were done with full consideration of animal welfare.

Results

Performance: Dietary supplementation of NB enzyme significantly ($P < 0.05$) increased body weight (BW) of poults fed the wheat-based diets, but there were only marginal response differences due to wheat source (Table 3). Poults fed the wheat-based diets supplemented with the enzyme had equal BW to those fed the corn-based control diet. Enzyme supplementation also significantly ($P < 0.05$) increased feed consumption (FC) among the poults fed the wheat-based diets (Table 4), although the birds fed the corn-based control diet had higher FC than those fed the wheat-based diets during the 1 to 17 days period (509.4 vs 467.9, $P < 0.05$). Enzyme supplementation improved feed conversion ratio (FCR) by about 4% ($P < 0.05$) for poults fed the wheat-based diets (Table 5). Poults fed the wheat-based diets had a significantly lower cumulative FCR (1 to 17 days) than poults fed corn-based diets (1.52 vs 1.72 g:g, $P < 0.05$). There were no significant differences found for wheat source or for the enzyme X wheat source interactions for BW, FC, or FCR. Cumulative livability averaged 99% for the entire experiment and was not significantly influenced by grain type (corn or wheat), wheat source, or enzyme supplementation (data not shown). In general, enzyme supplementation increased the growth performance of birds consuming the wheat-based diets to a level similar to those consuming the corn-based diets, regardless of source of wheat.

Table 4: Effects of wheat source[1] with (+) and without (-) enzyme (Natugrain Blend®[2]) supplementation on periodic feed consumption of poults raised from 0 to 17 days of age

Dietary Treatments		Days of Age			
Cereal Base	Enzyme	1-7	8-13	14-17	1-17
		(g)[3]			
Corn	-	103.1^{ab}	198.6	207.7^{a}	509.4^{a}
Wheat A	-	91.5^{cd}	203.1	185.1^{bcd}	479.7^{abc}
Wheat A	+	95.9^{bcd}	194.8	188.1^{bcd}	478.8^{abc}
Wheat B	-	88.8^{d}	179.0	174.7^{d}	442.5^{c}
Wheat B	+	98.0^{abc}	186.6	192.5^{abc}	477.1^{abc}
Wheat C	-	94.0^{cd}	180.7	176.5^{cd}	451.2^{c}
Wheat C	+	93.3^{cd}	184.4	192.9^{abc}	470.6^{bc}
Wheat D	-	90.6^{cd}	176.1	175.4^{d}	442.1^{c}
Wheat D	+	103.9^{a}	198.6	195.2^{ab}	497.7^{ab}
P-Value		0.0014	0.1251	0.0040	0.0094
SEM(36)[4]		2.62	7.50	5.82	13.35
Main effect of enzyme supplementation among wheat treatments				(g)[5]	
Enzyme -		91.2^{b}	184.7	177.9^{b}	453.9^{b}
Enzyme +		97.8^{a}	191.1	192.2^{a}	481.1^{a}
Source of variation among wheat treatments				(P-Value)	
Enzyme		0.0020	0.2266	0.0021	0.0085
Wheat		0.4488	0.1066	0.9670	0.4642
Wheat X enzyme		0.0828	0.2321	0.5009	0.2266
SEM(32)[6]		2.75	7.31	6.03	13.69

[a-d]Means with different superscripts within a column differ significantly ($P < 0.05$).
[1]The wheat sources differed by the degree of frost damage during seed development. Wheat A and B were frost damaged near full maturity, and wheat C and D were damaged during grain filling stage (mid-milk to soft-dough stage). [2]Natugrain Blend® contains 36,600 EXU/g of xylanase activity; 9,000 BGU/g of β-glucanase activity; and some hemicellulose, cellulase and protease activities (BASF, 1997). The dosage used was 200 mg/kg of feed, as recommended by the manufacture. [3]Values represent means of 5 cages containing 10 poults each. Data from 9 treatments (1 corn, 8 wheat). [4]SEM(36) = Standard Error of the mean with 36 degrees of freedom. [5]Values represent means of 20 cages containing 10 poults each. Data from 8 wheat treatments (4 wheat sources and 2 level enzyme). [6]SEM(32) = Standard Error of the mean with 32 degrees of freedom.

Ileum Digesta Viscosity: Jejunum digesta viscosity of birds fed the wheat-based diets without enzyme supplementation was significantly ($P < 0.05$) higher than for those fed the corn-based diets (Fig. 1). However, enzyme supplementation significantly reduced gut viscosity regardless of the source of wheat, such that it was statistically equivalent to the corn-based diets. Jejunum digesta viscosity was negatively correlated with AMEn, although the correlation coefficient was very low ($r = -0.22$, $P < 0.05$, Table 6). The negative correlation between jejunum digesta viscosity and ANR approached significance ($r = -0.20$, $P = 0.056$).

Energy and Protein Utilization (AMEn, ANR): The AMEn and ANR of birds fed the wheat-based diets were significantly lower than those fed the corn-based diets; however, enzyme supplementation significantly ($P < 0.05$) increased nutrient utilization, such that it was statistically equivalent to the corn-based diets (Table 6). The addition of NB to the wheat-based diets significantly increased the AMEn of wheat from 2,204 kcal/kg DM to 2,455 kcal/kg DM ($P < 0.001$). There was no wheat X enzyme interaction. However, there was a wheat source effect on nutrient utilization, where wheat A and B had significantly higher AMEn and ANR than wheat C and D (frost damaged during grain filling).

Discussion

This research compared the nutritional value of wheat that was frost-damaged during different stages of seed development, and how the nutritive value of such grain can be influenced by a blend of NSP-degrading enzymes. The commercial enzyme product used in this experiment, Natugrain Blend®, contained predominantly endoxylanase (36,600 EXU/g of product) along with β-glucanase (9,000 BGU/g of product), some hemicellulase (e.g. pectinase), cellulase, and protease activities (BASF, 1997). A blended preparation of enzymes was chosen because enzyme blends usually improves the nutritional value of wheat-based diets for monogastric animals more effectively than single enzyme preparations (White *et al.*, 1981; Odetallah *et al.*,

Table 5: Effects of wheat source[1] with (+) and without (-) enzyme (Natugrain Blend®[2]) supplementation on cumulative feed conversion ratio of poults raised from 0 to 17 days of age

Dietary Treatments		Days of Age		
Cereal Base	Enzyme	1-7	1-13	1-17
		(g/g)[3]		
Corn	-	1.200	1.578[a]	1.716[a]
Wheat A	-	1.312	1.522[a]	1.590[b]
Wheat A	+	1.172	1.362[b]	1.504[b]
Wheat B	-	1.258	1.342[b]	1.518[b]
Wheat B	+	1.166	1.348b	1.494[b]
Wheat C	-	1.246	1.384[b]	1.552[b]
Wheat C	+	1.182	1.326[b]	1.496[b]
Wheat D	-	1.232	1.398[b]	1.560[b]
Wheat D	+	1.184	1.338[b]	1.480[b]
P-Value		0.0912	0.0001	0.0047
SEM(36)[4]		0.035	0.036	0.039
Main effect of enzyme supplementation among wheat treatments		(g/g)[5]		
Enzyme -		1.262[a]	1.411[a]	1.555[a]
Enzyme +		1.176[b]	1.343[b]	1.493[b]
Source of variation among wheat treatments		(P-Value)		
Enzyme		0.0017	0.0133	0.0431
Wheat		0.7647	0.0512	0.7967
Wheat X enzyme		0.5899	0.1772	0.8730
SEM(32)[6]		0.035	0.037	0.041

[a,b]Means with different superscripts within a column differ significantly ($P < 0.05$). [1]The wheat sources differed by the degree of frost damage during seed development. Wheat A and B were frost damaged near full maturity, and wheat C and D were damaged during grain filling stage (mid-milk to soft-dough stage). [2]Natugrain Blend® contains 36,600 EXU/g of xylanase activity; 9,000 BGU/g of β-glucanase activity; and some hemicellulose, cellulase and protease activities (BASF, 1997). The dosage used was 200 mg/kg of feed, as recommended by the manufacture. [3]Values represent means of 5 cages containing 10 poults each. Data from 9 treatments (1 corn, 8 wheat). [4]SEM(36) = Standard Error of the mean with 36 degrees of freedom. [5]Values represent means of 20 cages containing 10 poults each. Data from 8 wheat treatments (4 wheat sources and 2 level enzyme). [6]SEM(32) = Standard Error of the mean with 32 degrees of freedom.

2002). When diets are supplemented with a blend of enzymes, the activity of one type of feed enzyme is facilitated by the activity of another (Ravindran *et al.*, 1999).

In the present experiment, enzyme supplementation significantly improved body weight (BW), feed consumption (FC), feed conversion ratio (FCR), dietary energy (AMEn) and the protein (ANR) utilization of poults fed wheat-based diets. These positive responses were attributed to the enzyme's ability to alleviate the adverse effects of excess dietary NSP. The poor performance of birds fed wheat-based diets that have not been supplemented with exogenous enzymes was attributed to NSP, mainly arabinoxylans, in the endosperm cell walls of the wheat kernel. As these NSP increase in the diet, nutrient digestion and absorption and growth performance decreases (Pettersson and Aman, 1989; Langhout *et al.*, 2000). However, supplementation of NSP-degrading enzyme preparations to cereal-based diets improves growth performance of monogastric animals, and this response has been associated with the reduction of viscosity in the intestinal tract (Choct *et al.*, 1996).

Our results confirm the hypothesis that the improvement from the enzymes in growth performance is mediated through a reduction in gut viscosity. Digesta viscosity was significantly higher in poults fed the wheat-based diet than those fed the corn-diet. However, enzyme supplementation to the wheat-based diets reduced jejunum viscosity to a level similar to that of birds fed corn-based diets. The reduction in digesta viscosity is associated with the improvement in the digestion of starch, protein, and lipids in the small intestine (Choct *et al.*, 1999). Similar results have been observed by other researchers who reported that endoxylanase improved dietary nutrient availability and increased performance (Bedford, 1995; Preston *et al.*, 2001).

Dietary endoxylanase supplementation elicits its beneficial effects on poultry by several means. Endoxylanase renders the xylose units more available to monogastrics (Odetallah, 2000). It also disrupts the water holding capacity of the NSP (Scott and Boldaji,

Table 6: Effects of wheat source[1] with (+) and without (-) enzyme (Natugrain Blend®[2]) supplementation on AMEn[8] and apparent nitrogen retention[9] (ANR) of poults[3]. And Pearson correlation coefficients (r) of the jejunum viscosity versus AMEn and ANR

Dietary Treatments		AMEn	ANR
Cereal Base	Natugrain Blend®	(kcal/kg)[3]	(%)[3]
Corn	-	2,582[a]	46.1[a]
Wheat A	-	2,291[bc]	37.4[cd]
Wheat A	+	2,501[ab]	45.3[ab]
Wheat B	-	2,297[bc]	39.1[bcd]
Wheat B	+	2,624[a]	46.0[a]
Wheat C	-	2,223[cd]	33.1[de]
Wheat C	+	2,270[c]	33.7[de]
Wheat D	-	2,005[d]	30.3[e]
Wheat D	+	2,428[abc]	40.8[abc]
P-Value		0.000[1]	0.0001
SEM (36)[4]		77.09	2.20
Main effect of enzyme supplementation among wheat treatments		(kcal/kg)[5]	(%)[5]
Enzyme -		2,204[b]	35.0[b]
Enzyme +		2,455[a]	41.4[a]
Main effect of wheat source among wheat treatments		(kcal/kg)[6]	(%)[6]
Wheat A		2,396[ab]	41.4[a]
Wheat B		2,460[a]	42.6[a]
Wheat C		2,246[bc]	33.4[b]
Wheat D		2,216[c]	35.6[b]
Source of variation among wheat treatments		---------- (P-Value) ----------	
Enzyme		0.0001	0.0002
Wheat		0.0098	0.0004
Wheat X enzyme		0.1158	0.1695
SEM(32)[7]		78.36	2.21
Correlation variable	Correlation coefficient (r)	----------	(P-Value) ----------
Viscosity vs AMEn	-0.2216		0.0358
Viscosity vs ANR	-0.2020		0.0562

[a-e]Means with different superscripts within a column differ significantly ($P < 0.05$).

[1]The wheat sources differed by the degree of frost damage during seed development. Wheat A and B were frost damaged near full maturity, and wheat C and D were damaged during grain filling stage (mid-milk to soft-dough stage).

[2]Natugrain Blend® contains 36,600 EXU/g of xylanase activity; 9,000 BGU/g of β-glucanase activity; and some hemicellulose, cellulase and protease activities (BASF, 1997). The dosage used were 200 mg/kg of feed, as recommended by the manufacture.

[3]Values represent means of 5-pooled samples of excreta per treatment collected from 11 to 14 days of age.

[4]SEM(36) = Standard Error of the mean with 36 degrees of freedom.

[5]Values represent means of 20 cages containing 10 poults each.

[6]Values represent means of 10 cages containing 10 poults each.

[7]SEM(32) = Standard Error of the mean with 32 degrees of freedom.

[8]Equation to determine AMEn, kcal/g diet on dry matter basis.

E-diet = kilocalories combustible energy per gram of diet dry matter (determined directly by bomb calorimeter)

E-excreta = kilocalories combustible energy in excreta per gram of diet dry matter =

= kilocalories per gram excreta x (g celite per gram diet/g celite per gram excreta)

N = Nitrogen retention per gram of diet dry matter =

=N per gram diet - N per gram excreta x (g celite per gram diet/g celite per gram excreta).

AMEn = Metabolizable energy per gram diet dry matter nitrogen corrected =

= E-diet - E-excreta - 8.22 N

[9]Equation to determine apparent nitrogen retention (ANR), %.

N-retained = Nitrogen retained per gram of diet dry matter =

= N per gram diet - N per gram excreta x (g Celite per gram diet/g celite per gram excreta)

ANR = Percentage of Apparent Nitrogen retention per gram of diet, percentage = (N-retained/N per gram diet) x 100

1997) and reduces the viscosity of the digesta in the small intestine (Bedford and Schulze, 1998; Choct *et al.*, 1999). Reduced digesta viscosity increases the diffusion rates of nutrients and endogenous enzymes, enabling the bird to digest and absorb more nutrients (Pawlik *et al.*, 1990). Endoxylanase releases entrapped nutrients for the digestion by the endogenous enzymes of the bird (Chesson, 2000). Therefore, dietary endoxylanase supplementation increases the performance and nutrient utilization of poultry by disrupting the gel-forming capacity of the NSP, which in turn enables better digestion and nutrient absorption in the intestinal tract.

Besides improving nutrient utilization by decreasing the viscosity, endoxylanase also improved AME and ANR by reducing enteric microflora fermentation. Langhout *et al.* (2000) reported that excess dietary NSP increased digesta viscosity, which caused changes in gut microflora and decreased nutrient digestion and absorption. In agreement, the data herein showed a significant decrease in AMEn as jejunum viscosity increased, even though a small negative correlation coefficient between jejunum viscosity and AMEn was present ($r = -0.22$, $P < 0.05$). Therefore, there are other factors that influence AMEn more than gut viscosity, such as microflora interaction on the antinutritive effect of wheat.

Endo-β (1,4)-D-xylanase is capable of hydrolyzing the xylan backbone of arabinoxylans to smaller fragments (Veldman and Vahl, 1994). A partial hydrolysis of β-glucan and arabinoxylan can reduce their water holding capacity and viscosity considerably. A reduction in gut viscosity can also increase feed passage rate and digestion, which can decrease the amount of indigestible material in the intestinal tract and decreases the proliferation of microflora in the small intestine (Bedford *et al.*, 1991; Van Paridon *et al.*, 1992). As the microflora is changed by enzyme supplementation, there is a decrease in the adverse effects of microbial fermentation. Adverse effects of microbial fermentation in the small intestine include: deconjugation of bile salts reducing fat digestion (Langhout, 1999), competition between the host and the microflora for nutrients (Bedford, 1995; Choct *et al.*, 1996; Langhout *et al.*, 2000), atrophy of the intestinal villi, and enlargement of digestive organs (Brenes *et al.*, 1993; Viveiros *et al.*, 1994). Therefore, xylanase addition could have increased nutrient digestion and performance of poults fed wheat-based diets by improving digesta viscosity and gut ecosystem characteristics.

The beneficial effect of low gut viscosity could be supported by our observations in the corn-diet group. This effect on the corn group could be associated with the influence of the enzymes on reducing digesta viscosity. Poults fed the corn-based diets had significantly higher FCR, although BW did not differ significantly from those fed the wheat-based diets. One reason for this observation could be that the birds with the corn-based diet had a lower gut viscosity, which allowed increased feed passage rate and thus increased feed intake. This observation is supported by the significantly higher FC among the birds fed corn-based diets (Table 4). Similarly, Antoniou *et al.* (1981) and Bedford and Classen (1992b) reported increased feed intake from enzyme supplementation of wheat-based diets, and they attributed this response to the enzyme's effect on reducing gut viscosity, and consequently increased feed passage rate.

There was no wheat source effect on BW, FC, FCR, viscosity, and mortality, even though wheat source effects were observed for AMEn and ANR (Table 6). Wheat A and B (frost-damaged near full maturity) had significantly higher nutrient digestibility than wheat C and D which were frost-damaged during the period of grain filling. The four wheat sources used in this study were similar in every aspect except planting time and the degree of maturation when they were frost damaged. All four wheat sources were from the same variety, grown on the same farm near Saskatoon, Saskatchewan, Canada. Therefore, the variation in response measurement among wheat sources due to the variety, geographical location and agronomic practices was minimized relative to the variation due to the degree of frost damage. Reports of the 1999 spring-wheat crop in Saskatchewan and many other regions in Canada showed significant loss of quality grade among frost damage and green immature kernels due to frost (Morgan, 1999).

The degree of frost damage depends on the stage of the maturation of the wheat kernels when the frost occurred. Wheat is tolerant to frost damage before the initiation of flowering, usually 8 to 10 weeks after germination when it can become dormant, as is the case with winter wheat varieties (Bendigo, 2000). However, wheat is very susceptible to frost damage during the period from the formation of its flowering parts to grain filling, i.e. the milk and dough stages (Bendigo, 2000). The development of the wheat kernel usually takes 12 to 21 days. The adverse effect of frost during the growth of the reproductive tissue and flowering is floret death and reduced yield. Frost damage during the grain filling, as occurred in the wheat C and D used in this experiment, can result in shriveled or shrunken grain that result low test weight, low falling number and high screening at harvest (Arnott and Richardson, 2001). Wheat kernels that reach full size and nearly full weight after the mid-dough stage, like the wheats A and B used in this experiment, are more resistant to freezing temperatures, and usually the only visible sign of frost damage may be a wrinkled appearance of the kernels and a slightly reduced test weight (Warrick and Miller, 1999).

Frost damage during the grain filling stage can change the chemical composition of the grain and its nutrient availability. Frost damage during grain fill may prevent a

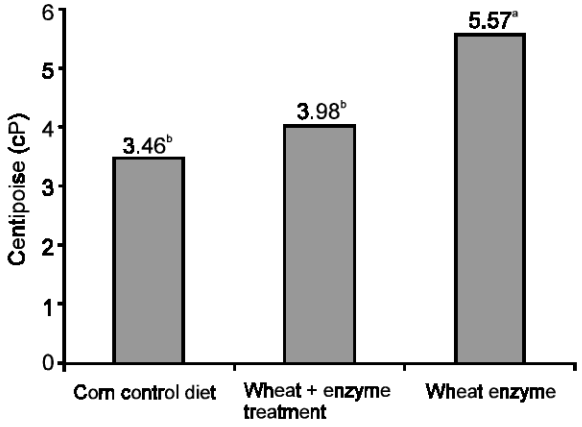

Fig. 1: Effects of wheat with (+) and without (-) enzyme (Natugrain Blend®) supplementation on the jejunum digesta viscosity of 17 days-old poults. Different letters on each bar signify a significant ($P < 0.05$) difference between mean values of jejunum digesta viscosity. Mean values represent average of two samples from twenty cages (corn had 5 cages) of 10 poults each. Natugrain Blend® contained 36,600 EXU/g of xylanase activity; 9,000 BGU/g of β-glucanase activity; and some hemicellulose, cellulase and protease activities (BASF, 1997). The dosage used was 200 mg/kg of feed, as recommended by the manufacture.

significant proportion of the sugars being converted to starch (Arnott and Richardson, 2001). The fiber and ash content may be higher in grain that is frost damaged during grain fill, due to a higher proportion of seed coat and a lower proportion of endosperm in the frost damage grain. Thus, frost damaged wheat may contain less gross energy and may have lower gross energy digestibility than normal wheat at full maturity. Furthermore, frost-damaged grains often contain higher levels of crude protein than normal grain because frost reduces the amount of starch deposited in the grain, thereby increasing the proportion of protein-to-starch (Arnott and Richardson, 2001).

There was very little variation in the chemical composition of the test wheats due to the degree of frost damage (Table 2). Although gross energy content did not differ significantly among wheat sources, the wheat frost-damaged during seed development had slightly higher crude protein, fiber and ash than the wheat exposed to frost during the ripening stage. The decrease in energy digestibility observed for wheats C and D support our hypothesis that frost damage during the grain filling stage adversely affects the total starch content in favor of more fiber. Moreover, the degree of maturity at harvest may have a significant influence on the overall nutritional value of wheat.

Frost damage during the milk-stage of seed development, as occurred in wheat C and D, arrests the synthesis and accumulation of starch into the wheat kernel and thus prevents the grain from fully maturing. Jennings and Morton (1963) and D'Appolonia and Mac Arthur (1975) reported that the pentosan/kernel in the endosperm increases throughout kernel development, as this is attributed to the synthesis of new cell walls that surround the newly synthesized starch. Starch synthesis starts early after pollination and continues until the grain kernel matures. Because immature wheat kernels contain less starch than kernels of more mature wheat, the NSP content is proportionally greater. Immature or frost-damaged wheat has a higher proportion of seed coat relative to endosperm than mature wheat. D'Appolonia and Mac Arthur (1975) reported that immature wheat has higher percentage levels of NSP, primarily arising from the bran (pericarp and testa) and endosperm layers. They observed that the arabinose:xylose ratio in the bran fraction was similar for both immature and mature wheat samples, but this ratio for the endosperm was higher in the immature than in the mature wheat. Therefore, immature wheat not only contains a higher amount of pentosan, but its pentosan is more water soluble than in mature wheat because the arabinose side chain is the water-soluble portion of the NSP (Ward, 1995). In addition, the NSP in immature wheat has a lower degree of branching than in mature wheat (D'Appolonia and MacArthur, 1975), which increases the hydration capacity and gel viscosity. The hydration capacity of polymerized arabinoxylans is dependent on the size of the molecules: water absorption increases as molecule size decreases (Izydorczyk *et al.*, 1991). The data from the present study further support the findings of D'Appolonia and MacArthur (1975), because wheat damaged at an immature stage had lower AMEn and ANR than normal wheat. The low AME found for the wheat that was frost-damaged at an immature stage is in agreement with findings of other researchers who reported different enzyme responses due to the high heterogeneity in water-soluble arabinoxylan content among wheat cultivars (Saulnier *et al.*, 1995; Crouch *et al.*, 1997).

A number of researchers have shown that enzyme supplementation reduces the variability in nutrient utilization among different sources of wheat. Choct *et al.* (1995) reported that enzyme supplementation significantly improved the nutritive value of a diet containing wheat with a particular low AME. Using broiler chickens, Scott and Pierce (2001) measured the feeding value of western Canadian wheat. They reported that variations in AME of wheat- or barley-based diets was significantly reduced by enzyme supplementation, indicating that low AME cereal grains generally benefit more from enzyme supplementation than high AME cereal grains. This is of great interest for the poultry

industry because not only does enzyme supplementation significantly improve nutrient utilization, but it also leads to more consistent uniform performance (Choct *et al.*, 1999). In our study, however, enzyme supplementation was equally effective for all of the wheat groups as indicated by growth performance, gut viscosity, and nutrient utilization. An insignificant enzyme X wheat effect may have been due to the fact that the wheat used in this study only differed by the degree of frost damage and there were no other genetic or agronomic differences.

From the results obtained, it can be concluded that supplementation of enzymes, as contained in Natugrain Blend®, improved the nutritional value of wheat-based diets to a level similar to diets containing corn in place of wheat. Frost damage during seed development significantly reduces the nutritional value of wheat, presumably by increasing the relative content of NSP to starch. This study demonstrated the positive effects of dietary enzyme supplementation on nutrient utilization of wheat grown under different wheat sources and cultivation conditions.

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[1]BASF AG, 67059 Ludwigshafen, Germany.
[2]Solka-Floc 40, Powdered cellulose, FS&D Fiber Sales and Development Corp, Urbana, OH, 43078.
[3]Pressure Sprayer 1 ½ quart, Delta Industries, North Hollywood, CA.
[4]Celite™, A diatomite product, Food Chemicals Codex Grade. Celite Corp., Lompar, CA 93436.
[5]British United Turkeys of America, Lewisburg, WV.
[6]Goldsboro Milling Company, Goldsboro, NC.
[7]National Wing Bands - Style 898, National Band & Tag Co., Newport, KY.
[8]Petersime Brood Unit, Model 2SD, Serial 2769, Petersime Incubator Company, Gettysburg, OH.
[9]Ziploc Gallon Bags, Johnson & Son, Inc., Racine, WI.
[10]Microcentrifuge Micro 13, Fisher Scientific, Pittsburgh, PA.
[11]Brookfield Engineering Laboratories Inc., Stoughton, MA.
[12]Waring Commercial Laboratory Blender, Model # 31BL91-7010, Torrington, CT.
[13]Blue-M, Model # DC-326F, Serial # DC-509, Blue M, Atlanta, GA.
[14]IKA Calorimeter System C5000 control, IKA® Werke Labortechnik, Staufen, Germany.
[15]KJELTEC Auto 1030 Analyzer, Tecator, Sweden.