The Use of Enzyme Technology for Improved Energy Utilization from Full-Fat Oilseeds. Part II: Flaxseed

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ABSTRACT An in vitro incubation study was carried out to determine whether various carbohydrase preparations contained appropriate activities to target nonstarch polysaccharides (NSP) of full-fat flaxseed. Enzyme preparations C (cellulase, 340 U/g), XG (xylanase, 63,600 U/g and glucanase, 48,300 U/g), P (pectinase, 10,000 U/g), and MC (mannanase, 10,900 U/g and cellulase, 600 U/g), alone and in combination (C + P, C + XG, P + XG, C + P + XG, C + P + MC, and C + P + XG + MC), were evaluated. Triplicate samples of defatted flaxseed meal (0.1 g) were incubated with 1% single enzymes or combinations at 45°C and pH 5.2. A more pronounced degradation of NSP was achieved when the enzyme preparations were used in concert. Compared with the control (no enzyme) treatment, the degree of NSP degradation averaged 34.7% when the sample was incubated with the 3 most effective enzyme combinations (C + P + XG, C + P + MC, and C + P + XG + MC). The effect of carbohydrase enzyme supplementation on energy utilization from full-fat flaxseed was investigated in a TME_n assay with adult roosters. When compared with the nonsupplemented sample, an increase ($P < 0.05$) in TME_n content from 2,717 to 3,751 kcal/kg (on average) was observed for the flaxseed supplemented with enzymes C + P + XG, C + P + MC, and C + P + XG + MC. A similar pattern of increase ($P < 0.05$) in fat and NSP digestibilities was noted. Enzyme combination C + P + XG was further evaluated in a 2-wk (5- to 18-d) trial with broiler chickens fed a corn and soybean meal-based flaxseed (15%) diet or the flaxseed diet supplemented with the enzyme at 3 different levels: 0.002, 0.01, and 0.05%. When supplemented at the highest level, the enzyme blend improved ($P < 0.05$) feed:gain, total tract DM, fat and NSP digestibilities, AME_n content, and ileal fat digestibility. No effect of enzyme supplementation, regardless of the level used, on ileal protein digestibility and digesta viscosity was observed. The results of the current study suggest that multiactivity carbohydrase enzyme supplements may be used as a means to improve energy utilization from full-fat flaxseed and, thus, enhance its feeding value for poultry.

Key words: flaxseed, energy utilization, enzyme, poultry

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INTRODUCTION

Full-fat flaxseed contains approximately 40% oil and 22% protein and is a valuable source of energy and protein in poultry diets. In recent years, it has become an attractive feed ingredient in Canadian poultry diets because of its high content of ω-3 unsaturated fatty acids (48 to 58% of the oil; Ajuyah et al., 1991), which can be deposited in the egg or meat products (Caston and Leeson, 1990; Ajuyah et al., 1991; Aymond and Van Elswyk, 1995) and can have a positive effect on human health (Hargis and Van Elswyk, 1993; Ferrier et al., 1995; Mayo et al., 1995). However, reduced energy use and depressed growth and feed efficiency have been observed when incorporating 10 to 20% ground flaxseed into broiler diets (Ajuyah et al., 1991; Lee et al., 1991; Alzueta et al., 2003). Lee et al. (1991) found that the use of equal portions of flax meal plus flax oil in place of flaxseed, significantly improved BW, feed efficiency, and dietary AME. Lee et al. (1995) reported that the TME_n of the ground flaxseed was considerably lower than its reconstituted meal-oil mixture (3,750 vs. 5,070 kcal/kg), and more energy-yielding material was excreted in birds fed flaxseed. The less-than-optimum energy use from full-fat flaxseed might be a result of limited oil availability, because in conventionally ground flaxseed, a substantial amount of oil may be encapsulated by the cell wall or nonstarch polysaccharides (**NSP**).

Conversely, Lee et al. (1991) observed a further depression in performance and energy use when the level of flax meal in the broiler diets increased from 6.5 to 13%. This indicates that the energy use from flaxseed by broiler chickens may also be influenced by the presence of some antinutritive compounds in the seed, such as mucilage, linatine, cyanogenic glycosides, trypsin inhibitors, or phytic acid (Madhusudhan et al., 1986). Mucilage is a mixture of branched-chain, water-soluble polysaccharides that is

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Table 1. Composition and calculated analysis of basal diet

Ingredient	(g/kg)
Corn (8.5% CP)	462.0
Soybean meal (43% CP)	319.0
Flaxseed (21.5% CP)	150.0
Canola oil	20.0
Limestone[1]	14.0
Dicalcium phosphate[2]	15.5
DL-Methionine	1.1
L-Lysine-HCl	0.4
Mineral premix[3]	5.0
Vitamin premix[4]	10.0
Chromic oxide	3.0
Total	1,000.0
Calculated analysis	
CP,[5] %	21.0
AME, kcal/kg	3,003.0
Lysine, %	1.12
Methionine, %	0.49
Methionine + cystine, %	0.84
Calcium, %	0.96
Available phosphorus, %	0.43

[1]Contained 38% calcium.

[2]Contained 21% calcium and 18% phosphorus.

[3]Mineral premix provided per kilogram of diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.36 mg; Na, 1.6 g.

[4]Vitamin premix provided per kilogram of diet: vitamin A, 8,250 IU; vitamin D_3 1,000 IU; vitamin E, 11 IU; vitamin B_{12}, 0.012 mg; vitamin K, 1.1 mg; niacin, 53 mg; choline, 1,020 mg; folic acid, 0.75 mg; biotin, 0.25 mg; riboflavin, 5.5 mg.

[5]Calculated based on analyzed values.

present in the hulls of flaxseed (3 to 9% of the DM of the seed; Mazza and Biliarderis, 1989) and increases the viscosity of the intestinal contents of broilers (Rodriguez et al., 2001). The viscous properties of mucilage have been suggested to be a major factor in the antinutritive effects of flaxseed for broilers (Alzueta et al., 2003).

Carbohydrase enzymes have been used to target cell wall NSP of feedstuffs in poultry diets. Studies carried out in our laboratory have demonstrated that carbohydrase enzymes with appropriate cell wall-degrading activities were effective in depolymerizing cell wall NSP of canola meal and in facilitating energy (or fat) use from full-fat canola seed (Meng et al., 2005, 2006). However, the effectiveness of carbohydrase enzymes on energy use from full-fat flaxseed has not yet been investigated. Therefore, the objectives of the current research were to screen several carbohydrase preparations for their ability to degrade the NSP of flaxseed in vitro and to evaluate the effectiveness of selected enzyme combinations in improving energy use from full-fat flaxseed when fed to adult roosters (TME_n assay) and broiler chickens.

MATERIALS AND METHODS

In Vitro Enzyme Evaluation

An in vitro incubation study was carried out to determine whether various carbohydrase preparations contained appropriate activities to target NSP of flaxseed. The sample of flaxseed was ground to pass thorough a 1-mm sieve, defatted with hexane for 6 h, and air-dried for use in the assay. Enzyme (Canadian Bio-Systems Inc., Calgary, Alberta, Canada) preparations **C** (cellulase, 340 U/g), **XG** (xylanase, 63,600 U/g and glucanase, 48,300 U/g), **P** (pectinase, 10,000 U/g), and **MC** (mannanase, 10,900 U/g and cellulose, 600 U/g), alone and in combination (C + P, C + XG, P + XG, C + P + XG, C + P + MC, and C + P + XG + MC), were evaluated using defatted flaxseed as the substrate. The in vitro incubation procedure applied in this study was as described by Meng et al. (2005). Briefly, triplicate samples (0.1 g) of the flaxseed meal were incubated with a total of 1% single enzyme or enzyme combination in a 0.1 *M* sodium acetate buffer (pH 5.2) at 45°C for 16 h. In the case of enzyme combinations, an equal portion of each enzyme (i.e., 1:1, wt/wt) was used. The samples were then subjected to NSP analysis as described by Englyst and Cummings (1988) with some modifications (Slominski and Campbell, 1990). The degree of cell wall polysaccharide degradation was indicated by a reduced recovery of total NSP and their constituent sugars compared with the control treatment. Effective enzyme combinations were selected for further evaluation in vivo.

TME_n Assay

The effect of the 3 most effective enzyme combinations, C + P + XG, C + P + MC, and C + P + XG + MC, on energy use of full-fat flaxseed was evaluated in a TME_n assay. The sample of flaxseed used in the study was hammer-milled to pass through a 2-mm sieve using a Wiley mill standard model No. 3 grinder (Arthur H. Thomas Company, Philadelphia, PA). Enzymes were included in the flaxseed samples at a level of 0.1%, and an equal portion of each enzyme (i.e., 1:1, wt/wt) was used for each enzyme combination. Two commercial samples of flaxseed products were also evaluated and included a hammer-milled flaxseed and an extruded sample of a mixture of flaxseed (60%) and other ingredients. The 2 samples were obtained from feed manufacturers in Manitoba and Quebec, Canada and were reported to be used in laying hen rations for ω-3 egg production.

Nitrogen-corrected true metabolizable energy content and fat and NSP digestibilities of flaxseed without or with enzyme supplementation were determined using the assay procedure described by Sibbald (1986) with some modifications (Zhang et al., 1994). Briefly, each seed sample was precision-fed (25 g per bird) to 3 groups of 7 individually caged, mature Single Comb White Leghorn roosters following a 28-h fast. During the next 48 h, the excreta from each bird were collected. The excreta samples were frozen, freeze-dried, weighed to determine total output, ground to pass through 1-mm sieve, and pooled for each group for analysis of gross energy, nitrogen, fat, and NSP contents. Pooled excreta from 10 birds fed 50 mL of a 50% glucose solution (25 g of dry glucose) were used to determine the endogenous excretion of energy and nitrogen.

Broiler Chicken Performance and Nutrient Digestibility Study

The effect of the enzyme combination was further evaluated in a growth performance and nutrient digestibility experiment with broiler chickens. The dietary treatments included a corn and soybean meal-based, full-fat flaxseed (15%) diet and the full-fat flaxseed diet supplemented with enzyme C + P + XG at 3 levels: 0.002, 0.01, and 0.05%. The seeds were ground to pass through a 2-mm sieve as described in the TME_n assay section. The composition of the basal diet is shown in Table 1. Chromic oxide (3.0 g/kg) was included in the diets and was used to calculate nutrient digestibilities and AME_n content.

One-day-old male Arbor Acres broiler chicks were obtained from a local commercial hatchery. The birds were held in electrically heated Jamesway battery brooders (James Mfg. Co., Mount Joy, PA) for a 4-d preexperimental period and fed commercial chick starter crumbles (21% CP). On d 5, birds were fasted for 4 h, individually weighed, and randomly distributed among treatments using 5 birds per pen and 8 replicate pens per treatment. All diets were fed in a mash form for the 2-wk experimental period (5 to 18 d of age). The birds had free access to water and feed and were provided with continuous light. Body weight and feed intake were monitored weekly with pen as the experimental unit. Mean weight gain, feed intake, and feed-to-gain ratio were used to determine performance.

At the termination of the experiment (on d 18), excreta samples from each pen were collected over a 3-h period and immediately frozen at –20°C. The samples were then freeze-dried and finely ground for analysis of gross energy, nitrogen, DM, fat, NSP, and chromic oxide contents. Nitrogen-corrected apparent metabolizable energy contents and total tract digestibility of DM, fat, and NSP were calculated. On d 19, 24 birds (3 birds per pen) were randomly selected from each treatment group and killed by cervical dislocation. The contents of the jejunum (from the end of the duodenum to Meckel's diverticulum) and ileum (from Meckel's diverticulum to 1 cm above the ileocecal junction) were collected and pooled for 4 birds to yield 6 replicate samples per treatment. Fresh digesta (1.5 g) from the jejunum were centrifuged at 9,000 rpm for 10 min, and viscosity of the supernatant was determined at 40°C using the Brookfield digital viscometer (model DV-II+LV, Brookfield Engineering Laboratories, Stoughton, MA). The ileal digesta samples were frozen, freeze-dried, ground, and analyzed for fat, nitrogen, and chromic oxide to determine fat and protein digestibilities.

All animal procedures were conducted according to the guidelines of the Canadian Council on Animal Care; the protocol for this study was approved by the animal care committee of the University of Manitoba.

Chemical Analysis

Feed, digesta, and excreta samples were analyzed for chromic oxide using the procedure described by Williams et al. (1962). Nitrogen was determined by the combustion method using the LECO model FP 2000 combustion analyzer (LECO Corp., St. Joseph, MI), and the protein contents were calculated using the multiplication factor of 6.25. Gross energy was determined by bomb calorimetry using a Parr 1261 adiabatic calorimeter (Parr Instruments Co., Moline, IL). Fat was analyzed using the AOAC method 920.39 (AOAC, 1990). For DM determination, samples were dried in a forced-draught oven at 105°C for 6 h; NSP were determined as described previously.

Calculations and Statistical Analysis

In the performance and nutrient utilization study, the following equations were used for calculation of apparent total tract digestibility of DM, fat, and NSP (using NSP digestibility calculation as an example); ileal digestibility of fat and protein (using fat digestibility calculation as an example); and AME_n content of experimental diets (Hill et al., 1960):

$$\text{total tract NSP digestibility (\%)} = \{1 - [(Cr_2O_{3\ \%\ \text{diet}}/Cr_2O_{3\ \%\ \text{excreta}}) \times (NSP_{\%\ \text{excreta}}/NSP_{\%\ \text{diet}})]\} \times 100;$$

$$\text{ileal fat digestibility (\%)} = \{1 - [(Cr_2O_{3\ \%\ \text{diet}}/Cr_2O_{3\ \%\ \text{digesta}}) \times (fat_{\%\ \text{digesta}}/fat_{\%\ \text{diet}})]\} \times 100;$$

$$AME_n\ (\text{kcal/kg of diet}) = GE_{\text{kcal/kg diet}} - [GE_{\text{kcal/kg excreta}} \times (Cr_2O_{3\ \%\ \text{diet}}/Cr_2O_{3\ \%\ \text{excreta}})] - 8.22 \times \{N_{\%\ \text{diet}} - [N_{\%\ \text{excreta}} \times (Cr_2O_{3\ \%\ \text{diet}}/Cr_2O_{3\ \%\ \text{excreta}})]\}$$

where GE = gross energy, and 8.22 is the energy equivalent of uric acid nitrogen (i.e., 8.22 kcal/g of uric acid nitrogen).

All studies were set up as completely randomized designs, and data were subjected to ANOVA using the GLM procedure of SAS (SAS Institute, 1986). Means were separated using Duncan's multiple range tests (Snedecor and Cochran, 1980). All statements of significance are based on $P < 0.05$.

RESULTS AND DISCUSSION

The effects of single and combined carbohydrases on degradation of the cell wall polysaccharides of defatted flaxseed meal are presented in Table 2. Total NSP content of the flaxseed meal averaged 271 g/kg. Based on the measured fat content (42.5%) of the seed and <0.5% of fat content in the defatted seed meal, the total NSP content of the full-fat flaxseed used in the study was calculated to be 157 g/kg. Xylose (21%), glucose (29%), and uronic acids (23%) were found to be the major constituent sugars

Table 2. Degradation of nonstarch polysaccharides (NSP) following incubation of defatted flaxseed meal with different preparations of carbohydrase enzymes[1]

Enzyme	Component sugar						
	Rhamnose	Arabinose	Xylose	Galactose	Glucose	Uronic acids	Total NSP[2]
	(g/kg)						
None (control)	15.1[a]	27.0[a]	57.3[a]	27.0[a]	80.1[a]	62.7[a]	271[a]
Cellulase (C)	15.6[a]	21.9[c]	48.2[cd]	22.5[c]	57.8[c]	54.2[cde]	222[c]
Pectinase (P)	12.7[b]	22.9[bc]	50.4[bc]	23.2[bc]	72.4[b]	53.0[de]	236[b]
Xylanase and glucanase (XG)	15.3[a]	23.8[b]	52.9[b]	24.0[b]	72.2[b]	53.7[cde]	243[b]
Mannanase and cellulase (MC)	14.8[a]	19.0[ef]	45.7[d]	21.4[d]	55.9[cd]	59.8[bc]	218[c]
C + P	13.2[b]	21.5[cd]	46.4[cd]	21.5[d]	55.3[cd]	57.1[bcd]	217[cd]
C + XG	14.4[a]	19.3[e]	44.8[d]	20.0[e]	51.3[d]	56.4[cd]	208[d]
XG + P	12.1[bc]	21.6[cd]	48.4[cd]	21.7[d]	68.9[b]	50.8[ef]	225[c]
C + P + XG	11.9[bc]	17.7[fg]	40.4[e]	18.7[f]	45.6[e]	48.0[f]	184[fe]
C + P + MC	9.7[d]	15.9[h]	39.3[e]	16.7[g]	38.6[f]	47.0[f]	169[g]
C + P + XG + MC	11.2[c]	17.1[gh]	39.5[e]	18.4[f]	41.1[ef]	48.1[f]	178[fg]
SEM	0.39	0.50	1.24	0.27	1.46	1.50	3.13

[a–h]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]Means of triplicate determination.

[2]Includes mannose and fucose in addition to rhamnose, arabinose, xylose, galactose, glucose, and uronic acids.

followed by arabinose (10%), galactose (10%), and rhamnose (6%). This constituent sugar profile agrees well with those from other oilseed meals such as canola and soybean (Slominski and Campbell, 1990; Huisman et al., 1998; Meng et al., 2005), suggesting that pectic polymers, heteroxylans, and cellulose are the major polysaccharides of flaxseed. Mucilage is an important water-soluble polysaccharide in flax and has been reported to consist of a neutral arabinoxylan and an acidic pectic-like polysaccharide containing rhamnose, galactose, and galacturonic acid residues (Muralikirishna et al., 1987; Cui et al., 1994). It has been shown that the arabinoxylans are the major components responsible for the high viscosity of flax mucilage (Cui et al., 1994).

Incubation of flax meal with enzyme preparations C, P, XG, and MC resulted in a significant degree of NSP degradation that ranged from 10 to 20%. The constituent sugar profiles indicate that the reduced recoveries of total NSP observed for each of the enzyme preparations generally resulted from the proportional removal of all constituent sugars except rhamnose, the recovery of which was only reduced when enzyme P was included in the assay. When the enzymes were used in concert, a more pronounced degradation of flax NSP was achieved. When compared with the nonenzyme treatment, the degree of NSP depolymerization averaged 34.7% when incubated with 3 of the most effective enzyme combinations (C + P + XG, C + P + MC, and C + P + XG + MC).

The results of the TME_n assay are shown in Table 3. The hammer-milled, full-fat flaxseed showed an increase in TME_n content from 2,717 to 3,751 kcal/kg (on average) following supplementation with enzymes C + P + XG, C + P + MC, and C + P + XG + MC; there were no significant differences among the 3 enzyme supplements. The increase in energy availability was accompanied by a similar pattern of increase in apparent fat and NSP digestibilities in roosters receiving the enzyme-supplemented flaxseeds. It appears that the carbohydrase enzyme combinations used in the current study were effective in hydrolyzing the cell wall polysaccharides of flax, thereby removing the physical barrier for oil use from the oil-containing cells.

The TME_n value obtained in the present study for the ground flaxseed without enzyme supplementation (2,717

Table 3. Effect of different combinations of carbohydrase enzymes on digestibility of fat and nonstarch polysaccharides (NSP) and TME_n content of full-fat, laboratory hammer-milled flaxseed when fed to adult roosters[1]

Enzyme[2]	Fat	NSP	TME_n
	(%)		(kcal/kg)
None (control)	59.4[b]	12.9[b]	2,717[b]
C + P + XG	74.8[a]	35.8[a]	3,750[a]
C + P + MC	73.4[a]	37.3[a]	3,788[a]
C + P + XG + MC	74.5[a]	32.9[a]	3,714[a]
SEM	2.03	2.67	28.4

[a,b]Means within a column with no common superscripts differ significantly ($P < 0.05$).

[1]Means of 3 pooled samples of 7 birds each.

[2]Main enzyme activities in enzyme products: C = cellulase; P = pectinase; XG = xylanase and glucanase; MC = mannanase and cellulase.

Table 4. Effect of multicarbohydrase enzyme supplementation on available energy (TME_n) content of commercial samples of full-fat flaxseed products

Sample	TME_n
	(kcal/kg)
Flaxseed (hammer milled)	2,205[b]
Flaxseed (hammer milled) + enzyme[1]	3,300[a]
Flaxseed "mix" (60% flaxseed; extruded)	3,237[b]
Flaxseed "mix" (60% flaxseed; extruded) + enzyme[1]	3,610[a]

[a,b]Means within a column and each product with no common superscripts differ significantly ($P < 0.001$).

[1]Contained cellulase, pectinase, xylanase, and glucanase as main activities.

Table 5. Growth performance of broiler chickens (d 5 to 18) fed diets containing full-fat flaxseed supplemented with different levels of a multicarbohydrase enzyme[1]

Treatment	Feed intake	BW gain	Feed: gain
	(g per bird)		
Flaxseed diet	675.2	455.0	1.484[a]
Flaxseed diet + low-level enzyme[2] (0.002%)	684.6	466.9	1.466[ab]
Flaxseed diet + medium-level enzyme (0.01%)	680.3	464.8	1.463[ab]
Flaxseed diet + high-level enzyme (0.05%)	680.9	470.1	1.449[b]
SEM	10.7	7.9	0.010

[a,b]Means within a column with no common superscripts differ significantly ($P < 0.05$).

[1]Means of 8 replicate pens of 5 birds each.

[2]Contained cellulase, pectinase, xylanase, and glucanase as main activities.

kcal/kg) was much lower than the values of 3,774, 3,957, and 3,750 kcal/kg reported by Lee and Sim (1989), Barbour and Sim (1991), and Lee et al. (1995), respectively. As has been indicated for the canola seed study (Meng et al., 2006), such discrepancy in TME_n values between our study and the previous studies might have resulted from a finer grind of the seed used in the TME assays. Although the details of the grinding procedures used in the last 3 studies were not specified, it is a common practice in the energy evaluation assays to use a proper grinding procedure to ensure optimum energy use. Because a small amount of sample is usually needed to perform the TME assay, fine grinding using laboratory mills and/or coffee grinders is often the method of choice when evaluating the high oil-containing feedstuffs. However, under commercial conditions, higher diameter sieves (i.e., 4 mm) are often used for oilseed processing to avoid sieve plugging. When the seeds are premixed with cereal grains to overcome this problem, the grinding may still be insufficient for an effective rupture of seed structure. This was further substantiated in a trial in which commercial samples of flaxseed were subjected to energy evaluation. As illustrated in Table 4, neither conventional hammer milling nor extrusion of flaxseed was as effective in increasing the available energy content as dietary enzyme supplementation. It is of interest to note that the TME_n value (3,750 kcal/kg; on average) of the laboratory-ground flaxseed when supplemented with enzymes (Table 3) was similar to that reported in earlier studies, but was higher that that of the commercial sample of hammer-milled and enzyme-supplemented flaxseed (3,300 kcal/kg). This result may be indicative of incomplete rupture of the seeds during the conventional hammer milling process. It would appear evident from this study that following conventional hammer-mill grinding or extrusion, some portion of the oil may still be encapsulated by the cell wall structure, and enzyme supplementation could further facilitate the seed "digestion," which, in turn, would result in optimum energy use from full-fat flaxseed.

The growth performance of broiler chickens fed diets containing flaxseed without enzyme addition or supplemented with different levels of enzyme C + P + XG is shown in Table 5. Although feed intake and BW gain were not affected, the feed:gain of broilers fed the flaxseed diet was improved following supplementation with the enzyme blend at the highest level (0.05%). At the same high inclusion rate, enzyme supplementation resulted in a significant increase in apparent total tract digestibility of DM, fat, and NSP and AME_n content of the flaxseed-containing diet (Table 6). The results of ileal fat and protein digestibilities and jejunal digesta viscosity are presented in Table 7. Ileal fat digestibility was improved when the enzyme inclusion rate was 0.05%. However, no effect of enzyme supplementation, regardless of the level used, on ileal protein digestibility was observed. Digesta viscosity of birds fed the control diet was relatively high, and no viscosity reduction with enzyme supplementation was observed.

Poor feed:gain (1.484), low fat digestibility (56.4% at the ileal level), and low AME_n content (2,701 kcal/kg) were observed in the present study for the control diet. A

Table 6. Apparent total tract digestibility of DM, fat, nonstarch polysaccharides (NSP), and AME_n content of full-fat flaxseed diets fed to broiler chickens[1]

Treatment	DM	Fat	NSP	AME_n
	(%)			(kcal/kg of diet)
Flaxseed diet	62.0[b]	54.4[b]	14.5[b]	2,701[b]
Flaxseed diet + low-level enzyme[2] (0.002%)	63.7[ab]	55.3[ab]	15.2[b]	2,747[b]
Flaxseed diet + medium-level enzyme (0.01%)	63.4[ab]	56.8[ab]	17.2[ab]	2,770[b]
Flaxseed diet + high-level enzyme (0.05%)	64.8[a]	58.9[a]	20.4[a]	2,846[a]
SEM	0.65	1.40	1.57	25.4

[a,b]Means within a column with no common superscripts differ significantly ($P < 0.05$).

[1]Means of 6 pooled excreta samples of 5 birds each.

[2]Contained cellulase, pectinase, xylanase, and glucanase as main activities.

Table 7. Apparent ileal digestibility of fat and protein and jejunal digesta viscosity in broilers fed full-fat flaxseed diets[1]

Treatment	Fat	Protein	Digesta viscosity
	(%)		(mPa·s)
Flaxseed diet	56.4[b]	74.6	10.9
Flaxseed diet + low-level enzyme[2] (0.002%)	57.5[b]	73.6	10.4
Flaxseed diet + medium-level enzyme (0.01%)	61.8[ab]	76.2	9.8
Flaxseed diet + high-level enzyme (0.05%)	64.2[a]	77.9	9.4
SEM	2.01	2.02	0.44

[a,b]Means within a column with no common superscripts differ significantly ($P < 0.05$).

[1]Means of 6 pooled ileal digesta samples of 4 birds each.

[2]Contained cellulase, pectinase, xylanase, and glucanase as main activities.

marked depression in fat digestibility, AME_n, and growth performance has also been reported previously for broilers fed diets containing flaxseed (Ajuyah et al., 1991; Lee et al., 1991; Alzueta et al., 2003). Such inhibition of fat digestion might have resulted from the oil-encapsulating effect of the cell wall polysaccharides on one hand and the presence of mucilage and other antinutritive factors on the other hand. Alzueta et al. (2003) demonstrated that the adverse effects of including flaxseed in broiler diets were associated with a marked increase in viscosity of digesta, and the substitution of demucilaged flaxseed for flaxseed in the diet greatly reduced the intestinal viscosity and the antinutritive effects of flaxseed. In the present study, the observed high-intestinal viscosity indicates that flax mucilage, similar to the water-soluble and viscous β-glucan and arabinoxylan of cereal grains (Choct and Annison, 1992a; Brenes et al., 1993), may depress digestion and absorption of nutrients through increasing digesta viscosity because of its water solubility and gel-forming properties. This is supported by the markedly depressed fat digestibility observed in the present study. In this context, the impairment of fat availability caused by high gut viscosity has been reported to be the highest among the nutrients evaluated (Campbell et al., 1983; Choct and Annison, 1992b; Meng et al., 2004).

Because the digesta viscosity reduction with enzyme supplementation was minimal in the broiler chicken trial, the positive effect of carbohydrase enzyme supplementation was likely due to enhanced fat or energy use as a consequence of the reduced nutrient-encapsulating effect of the cell walls, similar to that observed in the TME_n study. As has been demonstrated for a canola seed-containing diet (Meng et al., 2006), a relatively high level of enzyme supplementation is needed to achieve a significant response in flaxseed-containing diets, possibly because of the structural complexity of the flax NSP. However, both broiler chicken performance data and nutrient digestibility values were lower than those observed earlier for diets containing canola seed (Meng et al., 2006). It appears that the enzyme cocktail effective in cell wall polysaccharide depolymerization (as demonstrated by the significant increase in NSP digestibility) was not effective in reducing the viscosity of flax mucilage, which might have inhibited fat digestion and absorption. Therefore, more research is needed to screen for more effective enzyme blends to reduce the viscosity of flax mucilage.

In conclusion, the present studies demonstrate that the multicarbohydrase preparation could be effective in degrading the cell wall polysaccharides and in improving energy use from flaxseed. Therefore, supplementation with cell wall-degrading enzyme supplements may serve as an attractive means of facilitating fat accessibility for digestion and thus enhancing the overall energy use and the feeding value of full-fat flaxseed for poultry.

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