Enzyme addition facilitates the post-disease compensatory growth of broiler chickens challenged with *Clostridium perfringens*

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Jia, W., Slominski, B. A., Bruce, H. L., Nyachoti, C. M. and Jones, R. O. 2009. **Enzyme addition facilitates the post-disease compensatory growth of broiler chickens challenged with *Clostridium perfringens*.** Can. J. Anim. Sci. **89**: 369–381. In vitro incubation studies using a multicarbohydrase supplement showed a significant depolymerization of nonstarch polysaccharides (NSP) of soybean meal (SBM), canola meal and flaxseed meal, which was associated with the production of water-soluble NSP hydrolysis products. Effects of diet type and enzyme addition on growth performance of broiler chickens were investigated in a *Clostridium perfringens* challenge (10^9 CFU bird^{-1} on day 14) study. A total of 2640 male chickens were assigned to six treatments (wheat/SBM, unchallenged; wheat/SBM challenged; wheat/SBM + enzyme, challenged; wheat/flaxseed, unchallenged; wheat/flaxseed, challenged; wheat/flaxseed + enzyme, challenged). When compared with the wheat/SBM-based diets, birds fed flaxseed-containing diets had a decreased final body weight, an inferior overall feed conversion ratio (FCR), and an increased intestinal digesta viscosity ($P < 0.01$). Pathogen challenge caused mucosal lesions and increased ($P < 0.05$) the incidence of necrotic enteritis mortality with the highest mortality rate observed in birds fed the flaxseed diet without enzyme. Enzyme addition decreased ($P < 0.05$) the overall FCR from 1.88 to 1.77 and from 1.96 to 1.86 in challenged birds fed wheat/SBM and flaxseed-containing diets, respectively. In conclusion, feeding flaxseed had adverse effects on growth performance, and enzyme addition improved the nutritive value of flaxseed-containing diets, and facilitated the post-disease compensatory growth of chickens after *C. perfringens* challenge.

Key words: Enzyme, flaxseed, *Clostridium perfringens*, broiler chicken

Jia, W., Slominski, B. A., Bruce, H. L., Nyachoti, C. M. et Jones, R. O. 2009. **L'ajout d'un enzyme facilite la croissance de compensation après la provocation des poulets de chair avec *Clostridium perfringens*** Can. J. Anim. Sci. **89**: 369–381. Les essais d'incubation *in vitro* qui recourent à un supplément fait de carbohydrases multiples révèlent une importante dépolymérisation des polysaccharides non amylacés (PNA) des tourteaux de soja (TS), de canola et de lin, avec pour résultat la synthèse des dérivés hydrosolubles de l'hydrolyse des PNA. Les auteurs ont examiné quels effets la ration et un supplément enzymatique avaient sur la croissance de poulets de chair provoqués avec *Clostridium perfringens* (10^9 UFC par volatile le 14^e jour). En tout, 2 640 sujets de sexe mâle ont été répartis entre 6 traitements (blé/TS, sans provocation; blé/TS, avec provocation; blé/TS + enzyme, avec provocation; blé/lin, sans provocation; blé/lin, avec provocation; blé/lin + enzyme, avec provocation). Comparativement aux rations blé/TS, les oiseaux nourris avec les rations renfermant du lin étaient moins gros à la fin de l'expérience, se caractérisaient par un indice de consommation inférieur et avaient des digest plus visqueux dans l'intestin ($P < 0{,}01$). La provocation avec la bactérie pathogène entraîne des lésions aux muqueuses et augmente ($P < 0{,}05$) le taux de mortalité par entérite nécrotique, le taux le plus élevé étant enregistré pour les oiseaux qui recevaient du lin sans enzyme. L'ajout d'un enzyme réduit ($P < 0{,}05$) l'indice de consommation de 1,88 à 1,77 et de 1,96 à 1,86, respectivement, chez les oiseaux qui avaient été provoqués et recevaient du blé/TS ou du blé/lin. En conclusion, le lin à un effet néfaste sur la croissance et l'addition d'un enzyme rehausse la valeur nutritive des rations qui en contiennent, tout en facilitant la croissance de compensation après provocation des poulets avec *C. perfringens*.

Mots clés: Enzyme, lin, *Clostridium perfringens*, poulets de chair

Necrotic enteritis (NE) is a disease observed in most poultry-growing areas of the world and it becomes increasingly prevalent with the removal of feed antibiotics (McDevitt et al. 2006). The causative agent, *Clostridium perfringens*, is a spore-forming anaerobic bacterium found in the intestinal tract of healthy birds, usually in low numbers (Barnes et al. 1972). Its rapid growth under certain conditions can lead to an outbreak of NE. Several dietary factors are known to predispose broiler chickens to NE including high levels of cereal grains rich in viscous polysaccharides

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(e.g., wheat, barley, rye) or animal protein supplements, including fish meal (Branton et al. 1987; Kaldhusdal and Hofshagen 1992; Riddell and Kong 1992; Truscott and Al-Sheikhly 1997). High intestinal viscosity caused by water-soluble cell wall/nonstarch polysaccharides (NSP) of cereal grains may impair dietary nutrient utilization (Gohl and Gohl 1977; Johnson and Gee 1981; Edwards et al. 1988; Fengler and Marquardt 1988; Ikegami et al. 1990; Choct and Annison 1992), and may result in a significant supply of nutrients for bacterial growth (Wagner and Thomas 1978; Choct et al. 1996), and, therefore, facilitate *C. perfringens* proliferation (Wagner and Thomas 1978; Smits et al. 1998; Langhout et al. 1999).

Carbohydrase enzymes have a direct, positive effect on animal performance by improving nutrient digestion and absorption, thereby reducing substrate availability for microbial growth in the ileum (Choct et al. 1999; Bedford and Apajalahti 2001). In the process of depolymerizing various polysaccharides in the diet, carbohydrase enzymes may produce galacto-, gluco-, manno-, or xylo-oligomers (Silva et al. 1983), which, similarly to prebiotics, may facilitate proliferation of bacteria beneficial for gut health such as *Bifidobacterium* and *Lactobaccillus* (Monsan and Paul 1995), thereby decreasing the growth of certain pathogenic species (Gibson and Roberfroid 1995). The use of lactic acid bacterial cultures *Lactobacillus acidophilus* and *Streptococcus faecalis* has shown promising results in suppressing *C. perfringens* proliferation (Fukata et al. 1991) and reducing *C. perfringens* associated mortality (Hofacre et al. 2003). Kiarie et al. (2008) reported that the NSP hydrolysis products obtained from soybean and canola meals had positive effects against infection of enterotoxigenic *Escherichia coli* in piglets. In addition, higher ileal lactobacilli counts and lactate content were found in piglets fed diets supplemented with a multicarbohydrase enzyme (Kiarie et al. 2007).

Incorporating flaxseed in poultry diets to produce n-3-enriched eggs or meat products has attracted interest of the poultry industry. However, depressed energy utilisation and growth performance have been observed in broiler chickens fed increased amounts of flaxseed (Ajuyah et al. 1991; Lee et al. 1991; Ortiz et al. 2001; Alzueta et al. 2003). This is associated with the presence of various anti-nutritional factors (ANF), including mucilage, which is a water-soluble polysaccharide and can markedly increase the intestinal viscosity in broiler chickens (Alzueta et al. 2003). However, whether or not feeding flaxseed affects the susceptibility of broiler chickens to NE has not yet been investigated.

Earlier research from this laboratory has demonstrated that a multicarbohydrase enzyme was effective in depolymerising NSP of flaxseed and canola seed thereby improving oil utilization (Slominski et al. 2006; Jia et al. 2008). Therefore, we hypothesize that the addition of a multicarbohydrase enzyme to broiler chickens diets would facilitate the production NSP hydrolysis products, which may exert some beneficial effects against NE outbreaks by promoting the growth of lactic acid bacteria and thus reducing *C. perfringens* proliferation.

The current study was undertaken to evaluate the effectiveness of enzyme addition on NSP depolymerization of flaxseed meal (FM), soybean meal (SBM) and canola meal (CM) and to investigate the effects of flaxseed and enzyme addition on growth performance and NE incidence in broiler chickens consuming practical diets and challenged with *C. perfringens*.

MATERIALS AND METHODS

In Vitro Study

NSP Depolymerization by a Multicarbohydrase Enzyme

A multicarbohydrase supplement used in the study contained cellulase, pectinase, xylanase, glucanase, mannanase and galactanase and was similar to that used in our earlier research on NSP depolymerization of SBM and CM (Meng et al. 2005; Meng and Slominski 2005) and flaxseed (Slominski et al. 2006). Soybean meal, CM, and FM ground to pass through a 1-mm sieve were used in the study. The incubation procedure applied in this study was similar to that described by Slominski and Campbell (1990), while nonstarch polysaccharides were determined by gas-liquid chromatography (component neutral sugars) and by colorimetry (uronic acids) using the procedure described by Englyst and Cummings (1988) with some modifications (Slominski and Campbell 1990). Briefly, 100-mg samples were boiled with 7 mL of 0.1 M sodium acetate buffer (pH 5.2) for 5 min and then incubated at 40°C in an environmentally controlled shaker with the enzyme supplement added at four different levels: 0.5, 1.0, 2.5 and 5.0% along with a buffer solution containing starch-degrading enzymes (amylase, pullulanase, and amyloglucosidase). Following incubation, ethanol was added to a final alcohol concentration of 80% and the mixture was left for 1 h at room temperature before being centrifuged. The supernatant was discarded and the dried residue was dissolved in 1 mL of 12 M sulfuric acid and incubated for 1 h at 35°C. Six milliliters of water and 5 mL of myo-inositol (internal standard) solution were then added and the mixture was boiled for 2 h. One milliliter of the hydrolysate was taken and neutralized with 12 M ammonium hydroxide, reduced with sodium borohydride, and acetylated with acetate anhydride in the presence of 1-methylimidazole. Component sugars were separated using a SP-2340 column and a Varian CP 3380 Gas Chromatograph. Uronic acids were determined using the procedure described by Scott (1979).

The degree of NSP depolymerization was indicated by a reduced recovery of total NSP comparing with the control treatment (without enzyme).

Characterization of NSP Hydrolysis Product

Soybean meal, CM and FM samples (30 g) were further subjected to a vigorous mixing with 400 mL of 80% ethanol to extract simple sugars, sucrose and oligosaccharides. The extraction was performed overnight in the environmentally controlled shaker and was repeated four times. After each extraction, the samples were subjected to centrifugation at $1990 \times g$ and the supernatant (ethanol) was discarded. After last extraction, the samples were dried under a fume hood for 2 d.

Ethanol-extracted (sugar-free) meals were used to determine the effect of enzyme addition on NSP depolymerization and the production of simple sugars, oligosaccharides and low molecular weight polysaccharides. To identify these products, any changes in the content of total NSP, water-insoluble NSP, water-soluble NSP, and monosaccharides following enzyme addition were monitored.

For simple sugar (monosaccharide) analysis, the ethanol-extracted meals (0.1 g) were incubated without or with enzyme addition (2.5 and 5.0%) at 40°C for 5 h in a 0.1 M sodium acetate buffer (pH 5.2) containing starch-degrading enzymes. Ethanol was then added to a final alcohol concentration of 80% and the mixtures were left for 1 h at room temperature. Following centrifugation, the supernatants were transferred to separate test tubes for simple sugar (monosaccharide) analysis, which involved ethanol evaporation, residue solubilization and sugar reduction, acetylation and GLC analysis as described above.

For water-insoluble NSP analysis, the ethanol-extracted meals (0.1 g) were incubated without or with enzyme addition (2.5 and 5.0%) at 40°C for 5 h in a 0.1 M sodium acetate buffer (pH 5.2) containing starch-degrading enzymes. Following centrifugation, the supernatants were discarded. To ensure the complete removal of water-soluble NSP, the pellets were washed with buffer, centrifuged, and the supernatants discarded. The dried residues were subjected to hydrolysis with 12 M sulfuric acid for 1 h at 35°C and the hydrolysis in 1 M sulfuric acid at 100°C for 2 h. The hydrolyzates were then neutralized with 12 M ammonium hydroxide, reduced with sodium borohydride, acetylated and the component sugars analyzed using the GLC methodology described above.

Water-soluble NSP were calculated by difference between the total and the water-insoluble NSP.

Broiler Chicken Study

Housing and Management

A total of 2640 male Ross-308 broiler chickens, vaccinated for Marek's disease and infectious bronchitis, were purchased from a commercial hatchery. The broiler chicken research facility at Nutreco Canada Agresearch in Burford, Ontario, Canada was used to conduct the study. Forty-eight pens were randomly assigned to treatment groups, with 55 birds per pen. Each pen provided 13.7 m^2 of floor space with a concrete floor and new chopped straw for bedding. A solid 31-cm-high plastic barrier separated adjacent pens. Precautions such as changing gloves and foot coverings between treatment pens were taken to avoid accidental contamination of unchallenged pens with the challenge organism. Lighting program, heating, ventilation and other management procedures were typical of broiler chicken producers in the local geographic area of Ontario, Canada. Water was provided by nipple-type drinkers and feed by trough-type feeders ad libitum. All animal procedures were conducted according to the guidelines of the Canadian Council on Animal Care (1993).

Experimental Design and Diets

A 2×3 factorial arrangement of treatments was used in a randomized complete block design to study the effects of diet type (wheat/SBM-based or flaxseed-containing diet, 120 g kg^{-1} of diet), enzyme addition and pathogen challenge (control, unchallenged; challenged; challenged with dietary enzyme addition). There were eight blocks of six pens per block, with one pen of each treatment in each block, and each replicate pen consisted of 55 birds for a total of 440 birds per treatment. The carbohydrase supplement was added at the rate of 1 kg per 1 tonne of feed and supplied 60 U cellulase, 1400 U pectinase, 1200 U xylanase, 800 U glucanase, 500 U mannanase, 30 U galactanase and other minor enzyme activities per kilogram of diet. Antibiotic- and coccidiostat-free diets were formulated to contain 3100 kcal kg^{-1} ME and 23% CP in the starter phase and 3100 kcal kg^{-1} ME and 20% CP in the grower phase (Table 1). All diets were pelleted and crumbled and pelleting temperature did not exceed 75°C.

Experimental Procedure, Sample Collection and Chemical Analysis

The experiment lasted for 37 d, consisting of two phases (0 to 21 d, starter; 21 to 37 d, grower-finisher). The *C. perfringens* challenge model used in this study was originally developed based on Prescott et al. (1978) and has been described in a number of publications (Brennan et al. 2001a, b). The *C. perfringens* strain used was originally isolated from a field case of NE in Ontario, and was known to produce lesions typical of NE with mild suppression of growth rate and minimal mortality. Feed was withdrawn from all birds for approximately 8 h before challenge. Inoculum was mixed with feed and the feed was offered to birds on the afternoon of day 14. Inoculation lasted for 16 h and the remaining inoculum-containing feed was weighed and discarded on the morning of day 15. Calculated inoculation dose ranged from 1.6×10^9 to 2.4×10^9 CFU per bird. During inoculation, control birds received their regular feed.

Feed consumption and body weight were measured on a pen basis on days 0, 14, 17, 21 and 37; whereas mortality was recorded daily. The dead birds were

Table 1. Composition and calculated analysis of experimental diets

Item	Wheat/SBM-based diets		Wheat/flaxseed-based diets	
	Starter	Grower	Starter	Grower
Ingredient (% of diet)				
Wheat	40.4	48.0	37.0	46.7
Barley	13.0	16.0	12.5	13.4
Soybean meal	22.4	12.0	18.5	8.0
Flaxseed	–	–	12.0	12.0
Canola meal	4.0	5.0	3.0	4.0
Porcine meat meal	5.0	5.0	5.0	5.0
Wheat middlings	8.0	8.0	8.0	8.0
Canola oil	4.90	3.90	1.86	0.83
Calcium carbonate	1.05	1.16	1.05	1.12
Dicalcium phosphate	0.33	0.08	0.28	0.07
Mineral and vitamin premix[z]	0.25	0.20	0.25	0.20
Salt	0.36	0.22	0.36	0.22
DL-Methionine	0.17	0.10	0.16	0.08
L-Lysine	0.03	0.20	0.04	0.25
Choline	0.08	0.06	0.08	0.06
Threonine	–	0.06	–	0.06
Calculated analysis				
CP (%)	23.1	19.9	22.8	19.7
ME (kcal kg^{-1})	3100.0	3100.0	3100.0	3100.0
Calcium (%)	1.02	1.00	1.03	1.00
Nonphytate phosphorus (%)	0.43	0.37	0.43	0.38
Sodium (%)	0.19	0.13	0.19	0.13
Methionine (%)	0.55	0.42	0.53	0.39
Methionine+cystine (%)	0.92	0.75	0.89	0.71
Lysine (%)	1.19	1.06	1.17	1.06
Threonine (%)	0.77	0.69	0.80	0.71

[z]Mineral and vitamin premix provided: Mn, 89 mg; Zn, 88 mg; Fe, 34 mg; Cu, 63 mg; Se, 0.3 mg; I, 1.8 mg; vitamin A, 6238 IU; vitamin D_3, 2275 IU; vitamin E, 20 IU; vitamin B_{12}, 0.013 mg; vitamin K, 2.9 mg; niacin, 75 mg; folic acid, 0.86 mg; biotin, 0.1 mg; riboflavin, 5.5 mg kg^{-1} of the starter diet and Mn, 71 mg; Zn, 71 mg; Fe, 27 mg; Cu, 50 mg; Se, 0.24 mg; I, 1.4 mg; vitamin A, 4990 IU; vitamin D_3, 1820 IU; vitamin E, 16 IU; vitamin B_{12}, 0.011 mg; vitamin K, 2.3 mg; niacin, 60 mg; folic acid, 0.69 mg; biotin, 0.08 mg; riboflavin, 4.4 mg kg^{-1} of the grower diet.

subjected to necropsy and the presence of intestinal lesions was used to diagnose whether the mortality was due to NE. Average daily feed intake, average daily gain and feed conversion ratio (FCR) were calculated for each period (days 0–14, 14–17, 17–21, 21–37, 0–37). On day 17, 16 birds per treatment (two birds per pen) were randomly selected and euthanized by asphyxiation with carbon dioxide. The small intestine from each bird was removed, opened and subjected to scoring for NE lesions by the same poultry pathologist using the following scale: 0, no gross lesions; 1, thin, friable small intestine; 2, focal necrosis and/or ulceration; 3, patchy necrosis; 4, severe, extensive mucosal necrosis (Johnson and Reid 1970; Prescott et al. 1978). The intestinal contents from jejunum and ileum were collected and samples from four birds were pooled to yield four replicates per treatment for bacteria enumeration. Subsamples (1.5 g from each pooled sample) were frozen in liquid nitrogen and stored at $-20^{\circ}C$ until needed for viscosity measurement. The thawed samples were centrifuged at $3600 \times g$ for 10 min, and viscosity of the supernatant was determined at $40^{\circ}C$ using the Brookfield digital viscometer (model DV-II+LV, Brookfield Engineering Laboratories, Stoughton, MA).

Bacteria Enumeration

Pooled digesta (10 g) were transferred into 90-mL sterile peptone and serially diluted. For *C. perfringens* enumeration, dilutions were plated on Perfringens agar base (OPSP, Oxoid Inc., Nepean, ON) containing supplements SR 76 and SR 7 (Oxoid Inc.) and were incubated at $38^{\circ}C$ for 48 h in jars containing gas generation kits (BBL GasPak Plus™, Becton Dickinson). Lactic acid bacteria were enumerated using MRS (de Man, Rogosa, Sharpe) agar (Difco, Detroit, MI) following incubation at $37^{\circ}C$ for 48 h. For coliform enumeration, dilutions were plated on Petrifilm™ Coliform and *E. coli* plates (3M Canada, Inc., London, ON). Typical colonies were counted following incubation at $35^{\circ}C$ for 24 h. Each sample was plated in duplicate.

Statistical Analysis

All the statistical analysis was conducted using the SAS program (version 9.1, SAS Institute, Inc., Cary, NC). In vitro data were tested by GLM procedure and means were separated by Tukey's Honestly Significant Difference (Steel et al. 1997). In the broiler chicken study, bacterial enumeration data were converted to $\log_{10}$ CFU g^{-1} before analysis. Performance parameters and lesion

scores were analyzed by the MIXED procedure due to the presence of a random effect (Block), whereas analyses based on pooled samples (bacterial numbers and viscosity) were tested by the GLM procedure (McLean et al. 1991). The fixed effects in the model included diet (wheat/SBM or wheat/flaxseed), treatment (control; challenge; challenge + enzyme), and the two-way interaction. Means were separated by Tukey's Honestly Significant Difference except for mortality data. Mortality was tested by the same model, but subjected to the FREQ and GENMOD procedures (Steel et al. 1997). The results were presented with actual frequencies with standard errors. The GENMOD analysis was performed using the binomial distribution and the logit function. All statements of significance are based on $P < 0.05$.

RESULTS

In Vitro Study

The total NSP contents of SBM, CM and FM averaged 142.6, 177.2 and 250.3 mg g^{-1}, respectively, with a significant degree of NSP degradation observed following incubation of the meals with an enzyme supplement (Table 2). When compared with the control samples (without enzyme), NSP degradation averaged 21, 30 and 20% for SBM, CM and FM, respectively, when 5.0% enzyme was used. The highest enzyme concentration (5.0%) resulted in the highest cell wall polysaccharide depolymerisation in SBM. However, there was no significant difference between 1.0 and 5.0% enzyme concentrations in flaxseed NSP degradation, and the depolymerization of canola NSP was similar for 2.5 and 5.0% enzyme levels.

The characterization of NSP and NSP hydrolysis products following incubation with a multicarbohydrase enzyme is presented in Fig. 1. The total NSP content of ethanol-extracted SBM averaged 179.7 mg g^{-1}, and consisted of 136.1 mg g^{-1} water-insoluble and 43.6 mg g^{-1} water-soluble NSP. Ethanol-extracted CM contained 227.2 mg g^{-1} total NSP, including 185.4 mg g^{-1} water-insoluble and 41.8 mg g^{-1} water-soluble NSP, whereas ethanol-extracted FM contained 188.7 mg g^{-1} water-insoluble and 157.9 mg g^{-1} water-soluble NSP, which resulted in a total of 346.6 mg g^{-1} NSP. After 5 h incubation with a multicarbohydrase enzyme, the amount of water-insoluble NSP in SBM numerically decreased ($P > 0.05$), whereas the content of water-soluble NSP and NSP hydrolysis products increased with some monosaccharides being released in the enzyme-treated samples. Among the two enzyme-treated SBM samples, no significant difference was observed in water-insoluble NSP or NSP hydrolysis products content; however, 5.0% enzyme concentration resulted in a higher release of monosaccharides. A similar response was found for CM and FM samples with enzyme addition resulting in reduced amounts of water-insoluble NSP and increased amounts of water-soluble NSP and NSP hydrolysis products. When compared with the 2.5% level, the use of 5.0% enzyme did not increase the degradation of water-insoluble NSP further, but produced more NSP hydrolysis products in CM. Larger amounts of monosaccharides were released in both samples at the 5% than at the 2.5% enzyme concentration level. Among the monosaccharides released, glucose, galactose and uronic acid were predominant in all meal samples (Table 3). The increase in total sugars associated with 5.0% enzyme concentration was due to an increase in glucose, galactose and mannose in SBM; glucose, galactose, arabinose and mannose in CM; and glucose, galactose and xylose in FM.

Table 2. Recovery of total nonstarch polysaccharides (NSP) from soybean meal (SBM), canola meal (CM) and flaxseed meal (FM) following incubation with a multicarbohydrase enzyme

	SBM	CM	FM
Enzyme dose		(mg g^{-1})	
None (control)[z]	142.6 ± 2.2*a*	177.2 ± 2.9*a*	250.3 ± 1.6*a*
0.5%	135.3 ± 1.8*ab*	153.9 ± 3.5*b*	211.1 ± 1.6*b*
1.0%	129.0 ± 1.8*b*	144.2 ± 3.5*bc*	202.3 ± 1.6*bc*
2.5%	119.5 ± 1.8*c*	127.4 ± 3.5*cd*	200.5 ± 1.6*c*
5.0%	112.9 ± 1.8*d*	124.0 ± 3.5*d*	200.1 ± 1.6*c*
P	< 0.001	< 0.001	< 0.001

[z]Values are means ± SEM, $n = 2$ except that $n = 3$ for enzyme treated SBM and CM control samples. *a-d* Means within a column with no common letter differ significantly ($P < 0.05$).

Broiler Chicken Study

Growth Performance

Before *C. perfringens* challenge (0–14 d), birds consuming wheat/SBM-based diets had greater feed intake and body weight gain than those consuming flaxseed-containing diets, and thus an improved FCR was observed regardless of experimental treatment (Table 4). After *C. perfringens* challenge (14–21 d), impaired body weight gain and FCR were observed in challenged birds consuming the wheat/SBM-based diet, whereas feed intake was not impacted. Among those fed flaxseed diets, feed intake (14–17 d) and body weight gain (14–21 d) decreased due to *C. perfringens* challenge, and an impaired FCR was observed in challenged birds consuming the flaxseed-containing diet during periods of 14–17 d and 17–21 d. In the grower phase (21–37 d), regardless of diet type, pathogen challenge no longer affected the feed consumption, and an increased body weight gain was observed in the challenged groups when compared with control birds. In the absence of enzyme, birds consuming wheat/SBM-based diets had similar FCR irrespective of pathogen challenge (wheat/SBM, unchallenged vs. challenged, 21–37 d). However, a decreased FCR was noted in challenged birds fed flaxseed-containing diet compared with the unchallenged birds during this period. Enzyme addition reduced the feed intake of challenged birds regardless

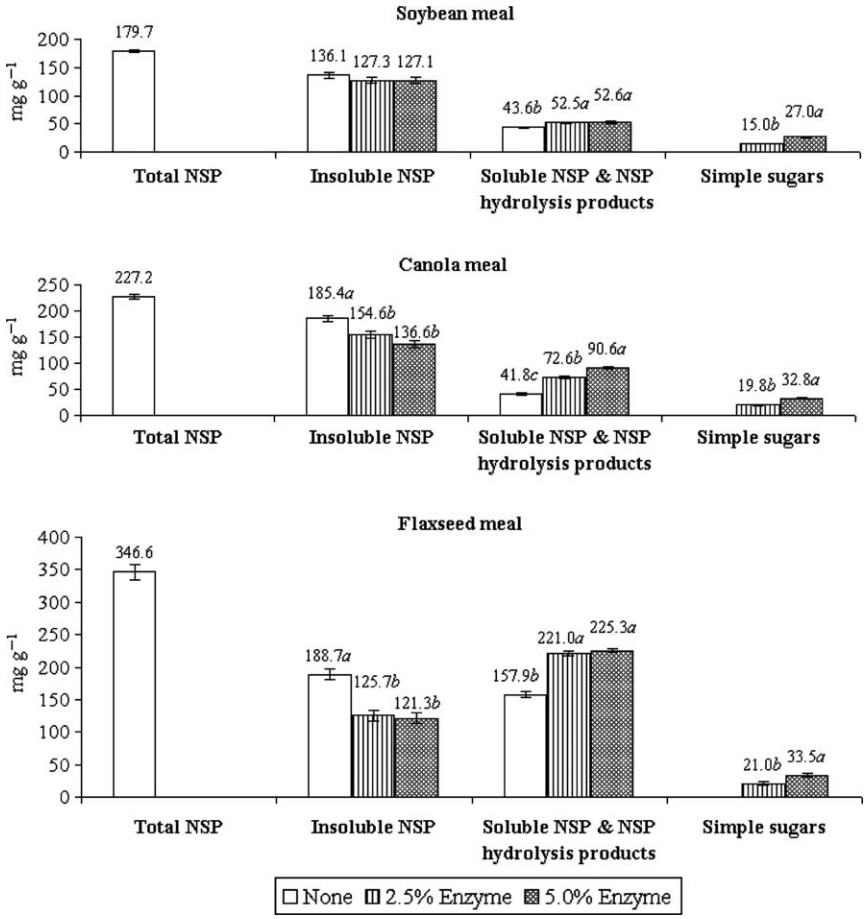

Fig. 1. Nonstarch polysaccharides (NSP) and NSP hydrolysis product balance after incubation of ethanol-extracted soybean meal, canola meal and flaxseed meal with a multicarbohydrase enzyme at 2.5% and 5.0% for 5 h. Soluble NSP and NSP hydrolysis products for the control samples represent water-soluble NSP only. Values are means ± SEM. $n = 3, 3, 3, 2, 3, 5, 3, 5, 3$, respectively, for each bar of soybean meal, $n = 2, 4, 4, 4, 2, 6, 5, 6, 5$, respectively, for each bar of canola meal, and $n = 2, 5, 5, 5, 2, 6, 5, 6, 5$, respectively, for each bar of flaxseed meal. *a,b* Means within a source with no common letter differ significantly ($P < 0.05$).

Table 3. Profile of monosaccharides released from ethanol-extracted soybean meal (SBM), canola meal (CM) and flaxseed meal (FM) following incubation with a multicarbohydrase enzyme

Treatment	Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acids	Total
	(mg g^{-1})						
SBM + 2.5% enzyme[z]	0.4 ± 0.2	–[w]	1.1 ± 0.2	3.6 ± 0.1	7.3 ± 0.3	2.5 ± 0.4	15.0 ± 0.6
SBM + 5.0% enzyme[y]	1.0 ± 0.3	–	2.2 ± 0.3	6.3 ± 0.2	14.0 ± 0.3	3.5 ± 0.5	27.0 ± 0.7
P	0.156	–	0.033	<0.001	<0.001	0.158	<0.001
CM + 2.5% enzyme[x]	2.7 ± 0.4	–	0.2 ± 0.1	1.2 ± 0.1	11.3 ± 0.5	4.3 ± 0.4	19.8 ± 1.1
CM + 5.0% enzyme[z]	4.9 ± 0.4	–	0.7 ± 0.1	2.1 ± 0.1	19.5 ± 0.5	5.6 ± 0.4	32.8 ± 1.2
P	0.004	–	0.007	<0.001	<0.001	0.053	<0.001
FM + 2.5% enzyme[x]	1.5 ± 0.2	0.0 ± 0.1	0.0 ± 0.1	1.9 ± 0.2	10.5 ± 1.0	7.1 ± 0.8	21.0 ± 2.2
FM + 5.0% enzyme[z]	2.1 ± 0.2	0.3 ± 0.1	0.1 ± 0.1	3.4 ± 0.3	18.7 ± 1.1	8.9 ± 0.9	33.5 ± 2.4
P	0.073	0.027	0.104	0.003	<0.001	0.156	0.004

[z]Values are means ± SEM. $n = 5$.
[y]$n = 3$.
[x]$n = 6$.
[w]Not detected.

Table 4. The effects of diet, enzyme addition and *C. perfringens* challenge on growth performance of broiler chickens[z]

	Average daily feed intake (g bird^{-1} d^{-1})				Average daily gain (g bird^{-1} d^{-1})				Feed conversion ratio (g of feed g^{-1} of gain)				
	0–14 d	14–17 d	17–21 d	21–37 d	0–14 d	14–17 d	17–21 d	21–37 d	0–14 d	14–17 d	17–21 d	21–37 d	0–37 d
Least squares means for diet × treatment													
Wheat/SBM, unchallenged	35.3*ab*	73.7*a*	93.6*a*	153.1	25.0*a*	45.7*a*	59.3*a*	75.8	1.42	1.41	1.71	2.02*bc*	1.82*d*
Wheat/SBM, challenged	37.2*a*	72.8*a*	97.3*a*	154.9	25.3*a*	39.6*b*	56.6*b*	76.2	1.47	1.59	1.96	2.04*bc*	1.88*c*
Wheat/SBM + enzyme,challenged	36.1*a*	71.1*a*	90.4*a*	148.3	25.5*a*	38.4*b*	55.9*b*	78.6	1.41	1.59	1.91	1.90*d*	1.77*d*
Wheat/flaxseed, unchallenged	34.1*b*	72.7*a*	97.1*a*	152.2	22.2*b*	45.0*a*	57.2*a*	66.9	1.54	1.42	1.86	2.33*a*	2.03*a*
Wheat/flaxseed, challenged	34.1*b*	64.9*b*	87.0*ab*	151.6	22.5*b*	31.5*c*	48.5*c*	72.7	1.52	1.75	2.25	2.10*b*	1.96*b*
Wheat/flaxseed + enzyme, challenged	32.1*b*	57.7*c*	78.6*b*	142.9	21.4*b*	30.4*c*	47.4*c*	71.6	1.50	1.63	1.97	1.99*cd*	1.86*cd*
SEM	0.5	1.5	2.5	2.2	0.2	1.2	1.0	1.1	0.02	0.04	0.10	0.03	0.02
Least squares means for main effects													
Wheat/SBM	36.2	72.5	93.8	152.1	25.3	41.2	57.3	76.9	1.43	1.53	1.86	1.99	1.83
Wheat/flaxseed	33.4	65.1	87.6	148.9	22.0	35.7	51.0	70.4	1.52	1.60	2.03	2.14	1.95
SEM	0.3	0.9	1.5	1.4	0.2	0.7	0.7	0.7	0.01	0.03	0.07	0.02	0.01
Control	34.7*ab*	73.2*a*	95.4*a*	152.7*a*	23.6	45.4*a*	58.2*a*	71.3*b*	1.48	1.41*b*	1.79*b*	2.18*a*	1.92*a*
Challenge	35.6*a*	68.9*b*	92.1*b*	153.3*a*	23.9	35.5*b*	52.6*b*	74.5*a*	1.50	1.67*a*	2.11*a*	2.07 *b*	1.92*a*
Challenge + enzyme	34.1*b*	64.4*c*	84.5*c*	145.6*b*	23.5	34.4*b*	51.6*b*	75.1*a*	1.46	1.61*a*	1.94*ab*	1.94*c*	1.82*b*
SEM	0.3	1.0	1.8	1.6	0.2	0.9	0.8	0.8	0.01	0.03	0.08	0.02	0.01
Factors and their significance based on MIXED analysis													
Diet	<0.001	<0.001	0.004	0.063	<0.001	<0.001	<0.001	<0.001	<0.001	0.019	0.020	<0.001	<0.001
Treatment	0.007	<0.001	<0.001	0.001	0.210	<0.001	<0.001	0.003	0.061	<0.001	0.002	<0.001	<0.001
Diet × treatment	0.020	0.001	0.007	0.539	0.004	0.002	0.004	0.061	0.087	0.057	0.335	<0.001	<0.001

[z]Two thousand six hundred and forty birds were assigned to six treatments in a randomized complete block design with eight blocks in total, 6 pens per block, and 55 birds per pen. Birds in challenged groups received an in-feed *C. perfringens* inoculation on day 14 that lasted for 16 h and the calculated dose ranged from 1.6 to 2.4×10^9 CFU bird^{-1}.
A–d Means within a column and within a source with no common letter differ significantly ($P < 0.05$).

of diet type in the grower phase, whereas the average daily gain was not affected, therefore FCR of both dietary groups decreased significantly following enzyme addition during this period (21–37 d). Over the entire trial (0–37 d), pathogen challenge resulted in an impaired FCR among birds consuming wheat/SBM diets (1.88 vs. 1.82), whereas an improved FCR was observed in those fed flaxseed-containing diets (1.96 vs. 2.03). Enzyme addition significantly improved the FCR of *C. perfringens* challenged birds in both dietary groups. With the inclusion of enzyme, the FCR of the challenged birds consuming the wheat/SBM diet was decreased to a level similar to that of unchallenged birds (1.77 vs.1.82, $P = 0.273$), with the FCR of birds fed the flaxseed-containing diet being even better than that of unchallenged birds (1.86 vs. 2.03, $P < 0.05$). In the absence of enzyme supplement, feeding flaxseed negatively affected the growth of birds, which was reflected by an increased FCR (0–37 d) and a decreased average final body weight (Table 5). Neither pathogen challenge nor enzyme addition influenced the final body weight.

Average Lesion Score and NE Mortality

No intestinal lesions were observed in unchallenged birds (Table 5). For those exhibiting lesions, they were scored as either focal or patchy necrosis. Neither diet type nor enzyme addition affected the average lesion score. *C. perfringens* challenge increased NE mortality from 1.1% to 3.2% in the wheat/SBM group and up to 7.7% in the flaxseed group (Table 5).

Bacterial Population and Viscosity of Intestinal Contents

Pathogen challenge and enzyme addition did not affect the intestinal *C. perfringens* numbers in birds consuming wheat/SBM-based diets (Table 6). Although the multiple comparison was not significantly different, contrast of mean *C. perfringens* numbers only in flaxseed groups showed that pathogen challenge significantly increased intestinal *C. perfringens* population from 2.3 in unchallenged birds to 4.3 $\log_{10}$ CFU g^{-1} in challenged birds ($P = 0.025$; not included in Table 6) in the absence of enzyme supplement. Similar *C. perfringens* counts of ileal digesta (i.e., 4.1 $\log_{10}$ CFU g^{-1}) were observed in broiler chickens 4 d after the challenge, and were lower than the number of 7.3 $\log_{10}$ CFU g^{-1} observed on day 1 post-challenge (Si et al. 2007).

Enzyme addition to the flaxseed-containing diet was accompanied by a 1.3 log reduction in *C. perfringens* counts (from 4.3 to 3.0 $\log_{10}$ CFU g^{-1}); however, this was not statistically significant. The numbers of total coliform and lactic acid bacteria were not influenced by any of the experimental treatments. A higher digesta viscosity was found in birds fed flaxseed diets than in those fed the wheat/SBM diets regardless of experimental treatment. Only a trend ($P = 0.06$) in viscosity reduction following enzyme supplementation was observed in birds fed flaxseed-containing diets.

Table 5. The effects of diet, enzyme addition and *C. perfringens* challenge on final body weight, average lesion score, and necrotic enteritis (NE) mortality in broiler chickens[z].

	Final body weight (kg, day 37)	Average lesion score	NE mortality (%)
Least squares means for diet × treatment			
Wheat/SBM, unchallenged	1.98 ± 0.02	-[y]	1.1 ± 0.5
Wheat/SBM, challenged	1.96 ± 0.02	0.6 ± 0.3	3.2 ± 0.8
Wheat/SBM + enzyme, challenged	2.00 ± 0.02	0.7 ± 0.3	5.4 ± 1.2
Wheat/flaxseed, unchallenged	1.79 ± 0.02	–	1.1 ± 0.5
Wheat/flaxseed, challenged	1.81 ± 0.02	0.9 ± 0.3	7.7 ± 1.3
Wheat/flaxseed + enzyme, challenged	1.77 ± 0.02	0.6 ± 0.3	5.2 ± 1.1
Least squares means for main effects			
Wheat/SBM	1.98 ± 0.01	0.6 ± 0.2	3.3 ± 0.6
Wheat/flaxseed	1.79 ± 0.01	0.7 ± 0.2	4.7 ± 0.5
Control	1.88 ± 0.01	–	1.1 ± 0.4*b*
Challenge	1.89 ± 0.01	0.7 ± 0.2	5.5 ± 0.8*a*
Challenge + enzyme	1.88 ± 0.01	0.6 ± 0.2	5.3 ± 0.8*a*
Significance of factors and their interaction from MIXED analysis (final body weight and lesion score) and Genmod analysis (mortality)			
Diet	< 0.001	0.732	0.252
Treatment	0.983	0.732	< 0.001
Diet × treatment	0.098	0.425	0.065

[z]Two thousand six hundred and forty birds were assigned to six treatments in a randomized complete block design with eight blocks in total, six pens per block, and 55 birds per pen. Birds in challenged groups received an in-feed *C. perfringens* inoculation on day 14 that lasted for 16 h and the calculated dose ranged from 1.6 to 2.4×10^9 CFU $bird^{-1}$. On day 17, two birds per pen were randomly selected and subjected to scoring for NE lesions using the following scale: 0, no gross lesions; 1, thin, friable small intestine; 2, focal necrosis and/or ulceration; 3, patchy necrosis; 4, severe, extensive mucosal necrosis. Values are means ± SEM.

[y]No intestinal lesions were observed in unchallenged birds.

a-c Means within a column and within a source with no common letter differ significantly ($P < 0.05$).

Table 6. The effects of diet, enzyme supplementation and *C. perfringens* challenge on bacteria numbers and viscosity of the intestinal contents[z]

	C. perfringens ($\log_{10}$ CFU g^{-1})	Coliforms ($\log_{10}$ CFU g^{-1})	Lactic acid bacteria ($\log_{10}$ CFU g^{-1})	Viscosity (mPa s^{-1})
Least squares means for diet × treatment				
Wheat/SBM, unchallenged	2.15±0.54	6.40±0.34	7.45±0.16	2.6±1.5
Wheat/SBM, challenged	2.96±0.54	6.58±0.34	7.59±0.16	2.9±1.5
Wheat/SBM+enzyme, challenged	2.61±0.54	5.56±0.34	7.39±0.16	2.1±1.5
Wheat/Flaxseed, unchallenged	2.29±0.54	5.50±0.34	7.42±0.16	11.0±1.5
Wheat/Flaxseed, challenged	4.32±0.63[y]	6.09±0.34	7.63±0.16	9.0±1.5
Wheat/Flaxseed+enzyme, challenged	2.96±0.54	6.20±0.34	7.55±0.16	4.8±1.5
Least squares means for main effects				
Wheat/SBM	2.57±0.31	6.18±0.20	7.48±0.09	2.5±0.9
Wheat/Flaxseed	3.19±0.33	5.93±0.20	7.53±0.09	8.3±0.9
Control	2.22±0.39	5.95±0.24	7.44±0.11	6.8±1.1
Challenge	3.64±0.42	6.33±0.24	7.61±0.11	5.9±1.1
Challenge+enzyme	2.79±0.39	5.88±0.24	7.47±0.11	3.5±1.1
Factors and their significance based on GLM analysis				
Diet	0.193	0.387	0.674	<0.001
Treatment	0.068	0.380	0.504	0.091
Diet × treatment	0.532	0.093	0.827	0.194

[z]Two thousand six hundred and forty birds were assigned to six treatments in a randomized complete block design with eight blocks in total, six pens per block, and 55 birds per pen. Birds in challenged groups received an in-feed *C. perfringens* inoculation on day 14 that lasted for 16 h and the calculated dose ranged from 1.6 to 2.4×10^9 CFU $bird^{-1}$. On day 17, two birds per pen were randomly selected, and intestinal contents from jejunum and ileum were collected and samples from four birds were pooled to yield four replicates per treatment for bacteria enumeration and viscosity measurement. Values are means±SEM.
[y]$n = 3$.

DISCUSSION

The use of carbohydrases to improve the nutritive value of viscous cereal-based diets has become a common practice in the feed industry (Cowieson et al. 2006). However, using enzymes to target flaxseed is a relatively new initiative and would appear to be more challenging (Slominski et al. 2006) because the composition of the cell wall polysaccharides in oilseeds is more complex than that of cereal grains (Chesson 2001). Pectic polysaccharides are predominant in dicotyledons, and they consist of a family of acidic polysaccharides (rhamnogalacturonans) and several neutral oligosaccharides and polysaccharides (arabinans, galactans, and arabinogalactans), which are believed to be covalently attached to the rhamnogalacturonan backbone (Bacic et al. 1988). It has been indicated that arabinogalactan and pectins are primary polysaccharides in the cotyledons of soybean, whereas the hulls contain cellulose, pectins, and hemicellulose polysaccharides, including galactomannan and xylan (Karr-Lilienthal et al. 2005). In addition to high concentration of pectins, non-cellulosic polysaccharides such as xylan, xyloglucan, arabinan, arabinogalactan and galactomannan are present in CM (Siddiqui and Wood 1977; Slominski and Campbell 1990). Although the cellulose content of FM is similar to that of SBM and CM (5.3% vs. 6.3% and 4.6–6.0%, respectively; Slominski and Campbell 1990; Bach Knudsen 1997), the total amount of cell wall polysaccharides in FM is much greater (Table 2), which is mainly due to the high content of water-soluble NSP (Fig. 1). There is a paucity of information regarding the composition of cell wall components in flaxseed except for mucilage. Mucilage is present in the outermost layer of the hull, and consists of two fractions, a neutral fraction composed of arabinoxylans and an acidic pectin-like fraction composed of polysaccharides containing rhamnose, galactose and galacturonic acid residues (Cui et al. 1994). The arabinoxylans are the major components responsible for the viscous property of mucilage (Cui et al. 1994). Poultry do not possess endogenous enzymes capable of cleaving and digesting cell wall polysaccharides such as cellulose, arabinoxylan, or pectic polysaccharides, therefore the nutritive value of flaxseed could be compromised. Results of the current study are in agreement with the literature data indicating that feeding flaxseed (12%) causes growth depression in broiler chickens (Ajuyah et al. 1991; Lee et al. 1991; Alzueta et al. 2003). When comparing the two unchallenged groups, birds fed the flaxseed-containing diet had inferior final body weight (Table 5) and overall FCR (Table 4) with no significant difference in feed intake when compared with those fed the wheat/SBM diet. This would indicate that the observed growth depression caused by flaxseed was related to the poorer nutrient utilisation.

Various ANF present in flaxseed, including mucilage, linatine, cyanogenic glycosides, and trypsin inhibitors, may contribute to the depressed nutrient utilisation (Bhatty 1995). The process of feed pelleting used in this study could inactivate some heat-labile components

(i.e., trypsin inhibitor, enzymes responsible for cyanogenic glycosides conversion to toxic end products); however, the mucilage and the NSP associated with the cell wall structure could still pose a problem and be responsible for growth depression. Oil is the main energy source in oilseeds, and is located in the cotyledon cells, which are surrounded by thick walls of polysaccharides. Because poultry cannot digest such polysaccharides, they create a physical barrier preventing oil from its full utilization. This rationale was substantiated in a rooster study in which the TME_n value of full-fat flaxseed was lower than the corresponding meal and oil mixture (3.7 vs. 5.1 kcal g^{-1}) (Lee et al. 1995). Furthermore, Ortiz et al. (2001) observed that increasing dietary flaxseed content in broiler chicken diets from 0 to 24% decreased the AME_n of the diet from 2799 to 2091 kcal kg^{-1}, which clearly indicate that, in addition to the oil encapsulating effect of cell walls, some flaxseed components may interfere with dietary nutrient utilization. Mucilage has been suggested to be responsible for the depressed growth rate and fat digestibility in broiler chickens by causing increased digesta viscosity (Alzueta et al. 2003). Although wheat is high in water-soluble arabinoxylan, the intestinal viscosity caused by mucilage present in the flaxseed-containing diets was much higher than that of wheat/SBM (Table 6).

Under the high intestinal viscosity, the substrate supply for microbial growth increases as a result of reduced nutrient utilization by host animals. Under such conditions, the rate of feed passage may increase (Salih et al. 1991), and mucus production may be enhanced (Larsen et al. 1993; Langhout et al. 1999; Piel et al. 2005). Such conditions may facilitate the overgrowth of ileal anaerobic bacteria, particularly of *Clostridia* (Choct et al. 1996; Smits et al. 1998; Langhout et al. 1999; Deplancke et al. 2002; Collier et al. 2003). Feeding viscous cereal-based diets (e.g., wheat and barley) has been indicated to be responsible for NE outbreaks in broiler chickens (Branton et al. 1987; Kaldhusdal and Hofshagen 1992; Kaldhusdal and Skjerve 1996; Annett et al. 2002; Jia et al. 2009). In the present study, the greatest values of NE mortality, intestinal *C. perfringens* number, and gut lesion score were observed in challenged birds fed the flaxseed-containing diet (Tables 5 and 6), which suggests that the viscous property of flax mucilage may have played some role. On the other hand, Allen et al. (1996) reported that the addition of n-3 fatty acids reduced cecal lesions and maintained body weight gains in broiler chickens infected with *Eimeria tenella*. If true, then α-linolenic acid in flaxseed may provide some protection against NE given that coccidiosis is an important risk factor in NE development (McDevitt et al. 2006). The current results do not conflict with this postulate, because no mucosal lesions could be found in randomly selected unchallenged birds consuming the flaxseed-containing diet, and the mortality and *C. perfringens* numbers were also minimal in this group, despite the high intestinal viscosity. In this context, it could be speculated that when birds are exposed to large numbers of *C. perfringens*, the high intestinal viscosity caused by flaxseed may overwhelm its potential protection and facilitate NE outbreaks. Therefore, the hygienic management of poultry farms, particularly those using flaxseed to produce n-3-enriched egg or meat products, is critical. Pathogen challenge did not affect the growth performance of birds during the grower phase (21–37 d), and superior body weight gain and FCR were noted in challenged birds fed the flaxseed-containing diet compared with the control birds (flaxseed, challenged vs. flaxseed, unchallenged). Considering the high mortality rate of this group, the better growth performance was due to the compensatory growth achieved by birds surviving the disease challenge.

The mode of action of carbohydrase enzymes is plant cell wall polysaccharide depolymerisation resulting in the production of water-soluble NSP and NSP hydrolysis products, including low molecular weight polysaccharides, oligosaccharides, disaccharides and monosaccharides (Silva et al. 1983; Pettersson and Aman 1989; Marsman et al. 1997). In the current study, incubation of the meals with a multicarbohydrase enzyme in vitro (Fig. 1) resulted in a significant decrease in water-insoluble NSP in CM and FM, which was associated with an increase in water-soluble NSP and NSP hydrolysis products including simple sugars. The relatively high concentrations of glucose and uronic acid residues (Table 3) produced by enzyme addition indicated, at least to some extent, that the hydrolysis of cellulose and pectic polysaccharides took place (Slominski and Campbell 1990), resulting in a disruption of intact cell walls. However, as demonstrated in Table 2 and Fig. 1, enzyme-specific activities rather than the enzyme concentration appears to be a limiting factor in achieving an effective NSP depolymerization. The in vitro study also demonstrated that the high production of water-soluble carbohydrate (soluble NSP and NSP hydrolysis products) due to enzyme addition to FM was followed by their minimal conversion, at least when compared with other meals, to simple sugars. This may explain why only a trend toward a reduction in digesta viscosity with enzyme supplementation was observed for birds fed the flaxseed-containing diets (from 9.0 to 4.8 mPa s, $P = 0.06$, Table 6).

Among the NSP hydrolysis products, galacto-, gluco-, manno- or xylo-oligomers could act as prebiotics and selectively stimulate proliferation of the gut health-promoting bacteria such as *Bifidobacterium* and *Lactobaccillus* (Monsan and Paul 1995), thereby decreasing growth of certain pathogenic species including *C. perfringens* (Fukata et al. 1991; Gibson and Roberfroid 1995; La Ragione and Woodward 2003). Kiarie et al. (2008) reported that carbohydrase hydrolysis products obtained from SBM and CM had positive effects against infection of enterotoxigenic *E. coli* in piglets. Higher ileal *Lactobacilli* counts and lactate content were found

in piglets fed enzyme-supplemented diets (Kiarie et al. 2007). In another study from this research group, broiler chickens were fed similar wheat/SBM-based and flaxseed-containing diets without or with a multicarbohydrase enzyme. Intestinal segments from birds were excised, ligated and inoculated with a *C. perfringens* spore cocktail. The results showed that enzyme addition significantly reduced the in vitro growth of *C. perfringens* in digesta from both dietary groups by 50 and 67%, respectively, with a more pronounced effect observed for the flaxseed group. This was followed by only a slight, but not statistically significant, increase in the growth of lactic acid bacteria (Wang 2008). However, only a numerical difference was observed in the current study with enzyme addition to the flaxseed-containing diet resulting in a 1.3 log reduction in *C. perfringens* numbers ($P > 0.05$, Table 6). A study from Australia (Choct et al. 2006) reported that xylanase supplementation reduced the *C. perfringens* numbers in the ileum and ceca of healthy broiler chickens fed low-ME (high viscosity) wheat. Because Canadian wheat is usually of lower viscosity, the better quality wheat used in the current study may have accounted for the lack of response from enzyme addition in terms of pathogen growth. Some literature reports demonstrated that enzyme addition stimulated growth of lactic acid bacteria in the small intestine (Vahjen et al. 1998; Engberg et al. 2004; Kiarie et al. 2007). However, numbers of lactic acid bacteria and coliform were unaffected by experimental treatments in the current study, which did not fully support our original hypothesis. Although not statistically significant, the trend toward numerical reduction of intestinal viscosity and *C. perfringens* number after enzyme addition to the flaxseed diet should not be ignored. It may, to a certain extent, reduce the risk of clinical outbreaks or the development of subclinical NE in broiler chickens under extensive rearing conditions. Further research in this area is needed.

Effects of enzyme on growth performance were most pronounced during the post-disease recovery period (21–37 d) in that enzyme addition significantly reduced FCR of challenged birds fed both diet types during the grower phase and over the entire experiment. Feeding flaxseed was associated with an inferior FCR, whereas after enzyme addition there was no significant difference in FCR between the two dietary treatments during the grower phase and over the entire trial (wheat/flaxseed + enzyme, challenged vs. wheat/SBM + enzyme, challenged) (Table 4), reflecting an enhanced dietary nutrient utilization and an improved nutritive value of flaxseed for poultry. This may lead to a reduced substrate supply for bacterial growth in the ileum (Choct et al. 1999; Bedford and Apajalahti 2001; Hubener et al. 2002), which, in turn, may potentially contribute to the control of *C. perfringens* growth and NE development in birds fed flaxseed-containing diets.

In conclusion, feeding flaxseed had adverse effects on growth performance of broiler chickens, and enzyme addition improved the nutritive value of flaxseed-containing diets, and facilitated the post-disease compensatory growth of birds after *C. perfringens* challenge. Although no significant changes in the incidence of NE were observed following enzyme addition, the numerical reduction of intestinal viscosity and *C. perfringens* numbers in birds fed high dietary level of flaxseed may, to some extent, lead to a reduced risk of clinical outbreaks or subclinical NE development without using antibiotic growth promoters.

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