IMPROVED NUTRIENT UTILIZATION AND GROWTH PERFORMANCE OF BROILER CHICKENS FED DIETS SUPPLEMENTED WITH MULTICARBOHYDRASE ENZYME PREPARATIONS

A Thesis

Submitted to the Faculty

of

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by

Xiangfeng Meng

In Partial Fulfillment of the

Requirements for the Degree

of

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Department of Animal Science

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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ABSTRACT

Among the feedstuffs commonly used in Canadian poultry diets, wheat, soybean meal (SBM), canola meal (CM), and peas contain significant amounts of nonstarch polysaccharides (NSP) which can be degraded by carbohydrases supplemenation. Low intestinal viscosity (2.0-5.0 mPa s) has been reported for broilers fed Canadian wheat-based diets. Supplementation of wheat-based diets with a single xylanase activity has often led to minor improvements in growth performance. In attempts to target the NSP in SBM, CM, and peas with carbohydrase enzymes, little response has been observed. Full-fat canola and flax seeds are valuable sources of energy, protein and α-linolenic acid for poultry diets. However, the energy utilization from these seeds is limited due to the oil encapsulating effect of the cell wall NSP. The objectives of the current research were (1) to screen various carbohydrase enzyme preparations in vitro for their ability to depolymerize the NSP of wheat, SBM, CM, peas, and flaxseed meal; and (2) to evaluated the effectiveness of selected enzyme combinations in improving nutrient utilization from wheat, SBM, CM, peas and full-fat canola and flax seeds when fed to broiler chickens. The in vitro studies demonstrated that a more pronounced degradation of NSP could be achieved when the enzyme preparations were used in concert. The selected enzyme combinations were evaluated further in a 2-wk growth performance and nutrient digestibility trial with broiler chickens fed a practical diet based on wheat, SBM, CM, and peas. All enzyme combinations were effective in improving ($P < 0.05$) growth performance, dietary AME_n, and starch and protein digestibilities of the birds with an intestinal viscosity being reduced from 3.3 to 2.3 mPa s. A combination of 4

carbohydrases was found to be superior ($P < 0.05$) to others in improving ileal protein digestibility and feed-to-gain ratio which were not due to a further reduction in digesta viscosity. When this enzyme combination was supplemented to 3 corn-based diets each containing 30% of either SBM, CM or peas, an improvement ($P < 0.05$) in NSP and protein digestibilities and dietary AME_n content was observed only for the SBM-containing diet. The performance of birds fed the 3 diets was not affected by the enzyme addition. The same enzyme blend was further used in a factorial experiment to evaluate if there is an interaction between fat type [beef tallow or canola oil (50 g/kg diet)] and carbohydrase addition [none or carbohydrases (0.4 g/kg diet)] in practical wheat-based broiler diets. Poorer performance and lower fat digestibility ($P < 0.001$) were noted for tallow-containing diets. Carbohydrase supplementation ($P < 0.001$) improved growth performance and nutrient digestibilities and reduced jejunal digesta viscosity for diets containing both fat types. The interaction between fat type and carbohydrase addition was only significant for fat digestibility values, with greater improvements seen for diets containing tallow. It appears that multicarbohydrase preparations could eliminate the negative effects of soluble NSP on animal fat utilization in a wheat-based broiler diet.

The effect of carbohydrase enzyme supplementation on energy utilization from full-fat canola and flax seeds was investigated in the TME_n assay and broiler experiments. In the TME_n assay, the TME_n and fat and NSP digestibilities increased markedly ($P < 0.05$) for both ground canola and flax seeds following supplementation with 3 different enzyme blends. One of the blends when supplemented at 0.05% to a corn-SBM based broiler diet containing 15% of canola seed or flaxseed showed an improvement ($P < 0.05$) in fat digestibility, dietary AME_n content, and feed-to-gain ratio.

However, no effect of enzyme supplementation on digesta viscosity was observed for birds fed flaxseed-containing diets. This data support the need for carbohydrase enzyme supplements in poultry diets containing full-fat canola and flax seeds.

FOREWORD

This thesis was written in manuscript format according to the guidelines of the Department of Animal Science, University of Manitoba. The manuscripts that have been published or submitted for publication are:

Manuscript 1. Meng, X., Slominski, B. A., Nyachoti, C. M., Campbell, L. D., and Guenter, W. 2005. Degradation of cell wall polysaccharides by combinations of carbohydrase enzymes and their effect on nutrient utilization and broiler chicken performance. Poult. Sci. 84:37-47.

Manuscript 2. Meng, X., and Slominski, B. A. 2005. Nutritive values of corn, soybean meal, canola meal and peas for broiler chickens as affected by a multicarbohydrase preparation of cell wall degrading enzymes. Poult. Sci. 84:1241-1251.

Manuscript 3. Meng, X., Slominski, B. A., and Guenter, W. 2004. Effect of dietary fat type, carbohydrase, and lipase addition on nutrient utilization and growth performance of young broilers fed wheat-based diets. Poult. Sci. 83:1718-1727.

Manuscript 4. Meng, X., Slominski, B. A., Campbell, L. D., and Guenter, W. 2005. The use of enzyme technology for improved energy utilization from full-fat oilseeds. Part I: Full-fat canola seed. Poult. Sci. (submitted)

Manuscript 5. Meng, X., Slominski, B. A., Campbell, L. D., Guenter, W., and Jones O. 2005. The use of enzyme technology for improved energy utilization from full-fat oilseeds. Part II: Full-fat flaxseed. Poult. Sci. (submitted)

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List of Abbreviations

AME – Apparent metabolizable energy

AME_n – Nitrogen corrected apparent metabolizable energy

BW – Body weight

C – Cellulase

CM – Canola meal

DM – Dry matter

G – Galactannase

LCSFA – Long chain saturated fatty acids

MC – Mannanase plus cellulase

NSP – Nonstarch polysaccharides

P – Pectinase

SBM – Soybean meal

TME – True metabolizable energy

TME_n – Nitrogen corrected true metabolizable energy

XG – Xylanase plus glucanase

1. INTRODUCTION

Carbohydrase enzymes, active against the nonstarch polysaccharides (NSP) in plant feedstuffs, are now extensively used in poultry diets throughout the world. The benefits in nutrient digestibility and growth performance may result from the enzymes' effects on reducing digesta viscosity (Annison, 1991; Bedford and Classen, 1992; Choct and Annison, 1992a) and / or disrupting cell wall structure (Pettersson and Aman, 1989; Chesson, 1993; Cowan et al., 1996). However, the mode of action of carbohydrase enzymes is still under debate. Further understanding of the mechanisms of enzyme action would lead to the development of more effective enzyme products for the feed industry.

Among the feedstuffs commonly used in Canadian poultry diets, wheat, soybean meal (SBM), canola meal (CM), and peas contain significant amounts of NSP. The positive effects of carbohydrases on wheat-based broiler diets have been attributed to the reduction in intestinal viscosity (Bedford and Classen, 1992). However, low intestinal viscosity (2.0-5.0 mPa s) has been reported for broilers fed Canadian wheat-based diets (Leeson et al., 2000; Slominski et al., 2000; McCracken and Miller, 2002) and the positive responses from enzyme addition are not always associated with a decrease in digesta viscosity (Veldman and Vahl, 1994; Dusel et al., 1998; Slominski et al., 2000; McCracken and Miller, 2002). Consequently, supplementation of wheat-based diets with a single xylanase activity has led to disappointing results (Chesson, 1993; Zhou, 2000) and the need for a multicarbohydrase preparation to further improve the nutritive value of wheat has been indicated.

In attempts to target the NSP in SBM, CM, and peas, less pronounced responses than those in cereal grains have been observed. Although carbohydrase enzymes appear to be beneficial in improving NSP digestibility of canola meal (Slominski and Campbell, 1990; Simbaya et al., 1996; Kocher et al., 2000) and in enhancing NSP, protein and energy utilization from SBM (Marsman et al., 1997; Douglas et al., 2000; Kocher et al., 2002), no beneficial effects on growth performance were observed. In the case of peas, no conclusive effects of supplemental carbohydrase enzymes on growth rate or feed-to-gain ratio in pea-fed broilers have been reported (Brenes et al., 1993a; Igbasan and Guenter, 1996; Igbasan et al., 1997b; Daveby et al., 1998).

It is a common practice in the Canadian feed industry to partially substitute CM and peas for SBM in wheat/SBM-based boiler diets for cost effectiveness. In such practical diets, which contain a number of plant ingredients and different forms of NSP, further improvements in nutrient utilization may be achieved by using combinations of carbohydrase enzymes, each differing in their substrate preference and mode of action, to target various cell wall polysaccharides. This will provide an avenue for further improvements in enzyme efficacy. However, the information on utilization of such enzyme combinations in practical broiler diets is limiting (Rosen, 2000).

Water-soluble viscous NSP of cereal grains (e.g. rye, wheat) have been shown to have a more pronounced negative effect on digestion of animal fats than vegetable oils by broilers fed diets containing high-viscosity grains (Danicke et al., 1997, 2000; Preston et al., 2001) due to ineffective emulsification of saturated fats and formation of micelles (Danicke et al., 1999c). Therefore, a significant interaction between fat type and enzyme supplementation has been demonstrated for broilers fed diets of high intestinal viscosity.

In addition, young birds do not digest and absorb fats effectively due to the age related availability of endogenous lipase and bile salts (Krogdahl and Sell, 1989; Noy and Sklan, 1995), both of which are essential for efficient micelle formation. High fat inclusion rates (e.g. 10%) and highly viscous semisynthetic diets (e.g. rye-based) have been used when fat type × enzyme interactions were evaluated (Danicke et al., 1997, 1999b, 2000). However, it is not clear if such interaction exists in practical broiler diets based on Canadian wheats of relatively low-viscosity.

Full-fat oilseeds such as canola and flax contain approximately 40% oil and 22% protein and are valuable sources of energy and protein for poultry diets. In recent years, they have become attractive feed ingredients in Canadian poultry diets because of their high content of omega-3 unsaturated fatty acids (8-12% and 48-58% of the oil for canola and flax, respectively) (Ajuyah et al., 1991), which can be deposited in the egg or meat products (Caston and Leeson, 1990; Ajuyah et al., 1991; Aymond et al., 1995) and consequently have a positive effect on human health (Hargis and Van Elswyk, 1993; Ferrier et al., 1995; Mayo et al., 1995). However, the optimum utilization of oil from full-fat canola and flax seeds by birds may be limited and in the conventionally ground seeds, substantial amounts of oil would be encapsulated by the polysaccharides of cell walls. The effectiveness of carbohydrase enzymes in eliminating the oil encapsulating effect and thus in improving energy utilization from full-fat canola and flax seeds has not yet been investigated.

The objectives of this research were:

1) To screen several carbohydrase preparations for their ability to depolymerize the NSP of wheat, SBM, canola meal, peas, and flaxseed meal in vitro;

2) To evaluate the efficacy of selected enzymes in growth performance and nutrient utilization studies with broilers fed practical diets based on wheat, SBM, CM, and peas;

3) To investigate the effectiveness of selected enzymes in improving the nutritive values of SBM, CM, and peas for broiler chickens;

4) To investigate if there is an interaction between dietary fat type, carbohydrase, and lipase supplementation in terms of nutrient utilization and growth performance in practical Canadian wheat-based broiler diets;

5) To evaluate the effectiveness of carbohydrase enzymes in improving energy utilization from full-fat canola and flax seeds when fed to poultry.

2. LITERATURE REVIEW

2.1. Nutrient Composition of Feedstuffs and the Components Affecting Their Utilization by Poultry

2.1.1. Wheat

Wheat is an important feed ingredient in poultry diets, contributing up to 70% of the metabolizable energy (ME) and 40% of the protein requirements of broilers (Hew et al., 1998). The nutrient composition and nutritive value of wheat vary considerably. Slominski (2000) evaluated 19 wheat samples collected from different feed manufacturers across Western Canada and found a range of 9.0 to 14.8% and 52.3 to 60.3% for protein and starch content, respectively. In addition, the variation in ME has been widely documented in the literature for wheat samples from different regions and countries. According to studies with broiler chickens, the ME contents of Australian wheat varied from 10 to 16MJ kg^{-1} DM (Mollah et al., 1983; Rogel et al., 1987; Hughes et al., 1996; Hughes and Choct, 1997). March and Biely (1973) reported ME levels between 12.6 and 15.9 MJ kg^{-1} DM in 33 samples of Canadian wheat obtained from a variety of sources. Similar variations, though of somewhat lower magnitude, have also been reported in other studies on Canadian wheat (Sibbald and Slinger, 1962; Scott et al., 1998; Slominski, 2000) and the UK wheat (McNab, 1991; Wiseman, 2000).

Poor growth performance was observed when the low-ME wheat samples were fed to broilers. Studies on a large number of Canadian wheats (March and Biely, 1973; Scott et al., 1998) indicated that the chicken growth rates were approximately 13% lower when fed the lowest-ME compared with the highest-ME wheat samples. A similar

magnitude of reduced growth rate has been observed in studies with UK wheat cultivars (Rose et al., 1993; McCracken and Quintin, 2000).

The nutritive value of wheat is affected by both variety and environment, but the intrinsic factors that cause varying nutritive value are not yet completely established. The ME value of wheat is not related to the presence of amylase inhibitors or the contents of protein and starch (Mollah et al., 1983). However, a strong positive correlation was found between ME and digestibility of starch (Mollah et al., 1983; Rogel et al., 1987). It was further demonstrated that starches isolated from wheat with low ME values were digested by chick pancreatic α-amylase in vitro to the same extent as starches from wheat with high ME values (Rogel et al., 1987). These data suggest that it is not starch per se that is poorly utilized in some samples of wheat. Subsequent research has indicated that NSP is the primary factor responsible for the low AME of some wheats (Annison, 1991; Scott et al., 1998; Choct et al., 1999). A strong negative correlation between wheat ME values and the levels of water-soluble NSP (i.e., arabinoxylans) has been documented (Annison, 1991). When isolated and added to broiler diets, wheat arabinoxylans caused a depression in nutrient utilization (starch, protein and fat), AME value and chick growth (Choct and Annison, 1990).

2.1.2. Corn

Having high available energy content and a relatively low protein content, corn has always been considered as the main energy source in poultry diets. However, since the levels of corn used in poultry diets can approach 70%, 20% of dietary protein content could be contributed by corn. While corn quality has always been assumed to be fairly consistent, some variations in its nutrient composition have been documented. Leeson

and Summers (1976) reported a crude protein content between 7.6 and 10.9% and a starch content between 55.3 and 58.2% for Canadian corn, which in addition varied from 3,051 to 3,300 kcal/kg in ME content as determined with adult roosters. Maier (1995) collected a number of samples from different parts of Indiana, USA and analyzed for various nutrients. A range of 5.7 to 9.7%, 59.9 to 64.8%, and 2.6 to 4.9% was observed for protein, starch, and oil content, respectively. It has been documented that such variable quality of corn could translate into variable bird performance and consequently impact on producer profitability (Bedford, 2001).

Harvesting conditions can have a significant effect on energy content. As an example, the 1992 harvest in Ontario, Canada, produced corn with ME values varying from 2,944 to 3,363 kcal/kg when weather conditions caused delays and the crops were harvested wet (Leeson et al., 1993). The differences in corn starch digestibility have been suggested to be a major factor contributing to the variability in ME content between different corn samples (Bedford, 2001). Young birds have a limited capacity to digest starch, with the undigested starch fraction (i.e., resistant starch, defined as the sum of starch derivatives not absorbed in the small intestine of healthy birds) of corn ranging from 2-6% (Weurding et al., 2001). The amounts of resistant starch in the diet may compromise the efficiency of feed utilization. The major limiting factor in the digestion of corn starch is the accessibility of substrate to digestive enzymes (Mateos et al., 2002). Starch accessibility is determined by factors such as the size and nature of the protective structures (i.e. NSP) surrounding the starch granules (Theander et al., 1989; Slominski et al., 1993; Bedford, 2002), and the structure of the starch. In general, starch in small granules has lower amylose content and is hydrolyzed more rapidly than starch in large

granules. Also, starches with low-amylose content are more digestible than starches with high-amylose content, because high-amylose starches are less susceptible to amylase action (Brown, 1996). In addition, A type starch granules (very compact with no space left for water) are easier to digest than the B (interior channel filled with water) or C type (intermediate) granules. While most cereals possess the A type, high-amylose corn possesses the B type granules, with the digestion rate being slower than in its conventional counterpart (i.e. A type). Protein encrustation of starch granules has also been suggested as a possible factor limiting exposure of starch to amylases (McAllister et al., 1993; Elkin et al., 1996).

2.1.3. Canola meal

Canola (the oil-free residue of low glucosinolate, low erucic acid rapeseed) meal is a good source of protein for poultry with the protein content ranging between 35 and 36% (CGC, 2000). It has a good amino acid profile for animal feeding and is particularly rich in the sulphur amino acids, methionine and cystine (NRC, 1994). Among the feedstuffs, CM is one of the richest sources of available phosphorus (NRC, 1994).

Canola meal is known to have a number of antinutritive factors that may affect nutrient utilization by poultry. However, the levels of glucosinolates and erucic acid, two of the more detrimental constituents of the canola varieties have been markedly reduced as a result of genetic selection. Erucic acid levels are now negligible while glucosinolate levels are down to less than 20 μg/g. These levels are low enough to be of little or no concern for poultry (Leeson and Summers, 2001). The tannin levels in canola can also be relatively high approaching 3% in some cultivars (Leeson and Summers, 2001). Research has shown that canola tannins have little influence on the utilization of protein, even in

diets containing appreciable levels of the meal (Mitaru et al., 1983). Phytic acid is present in CM in appreciable amounts (0.87%, NRC, 1994) and may affect nutrient utilization by binding phosphorus as well as other essential minerals and amino acids (Maenz, 2001).

Canola meal contains a relatively low level of ME (NRC, 1994) which is associated with high fiber and high NSP levels (Bell, 1993). It has also been demonstrated that overheating during commercial processing of canola can lead to losses in the content and availability of amino acids (Anderson-Hafermann et al., 1993; Newkirk et al., 2003). The decline in amino acid availability would lead to impaired chick performance (Anderson-Hafermann et al., 1993).

2.1.4. Soybean meal

Soybean meal is the most widely used vegetable protein in poultry dietS. Its amino acid profile is excellent for poultry, and when combined with corn, methionine is usually the only limiting amino acid. The protein level in SBM can be variable (44.0-48.5%, NRC, 1994), and this may be a reflection of variability among varieties and/or processing conditions of oil extraction (Dale, 1996).

Antinutritive factors present in raw soybeans (e.g. trypsin inhibitors, lectins, and saponins) are to a large extent destroyed during processing. However, excessive heating during processing may reduce the availability of some amino acids, particularly lysine and consequently impair growth performance (Parsons et al., 1992). Protein and amino acid digestibility may also be affected by the presence of high levels of phytic acid in SBM (Sebastian et al., 1997).

Soybean meal is also known for its low ME content. Only about 55% of the gross energy in SBM is available to the bird (Dale, 1996). This is largely due to the poorly

digestible carbohydrate fractions including oligosaccharides and NSP, for which birds lack the proper digestive enzymes. Soybean meal usually contains about 6% oligosaccharides, which are mainly α-galactosides: raffinose and stachyose. Raffinose and stachyose have been indicated to have a depressing effect on utilization of energy from SBM (Leske and Coon, 1999). The meal from genetically modified soybeans with low levels of α-galactosides has been shown to have higher TME_n content than the meal from conventional soybeans (Parsons et al., 1996). Coon et al. (1990) and Leske et al. (1993) reported that removal of oligosaccharides using ethanol extraction resulted in increased TME_n in SBM. However, the nutritional implications of dietary α-galactosides have been contradictory. Angel et al. (1988) and Irish et al. (1995) found no significant difference in growth performance of broilers fed SBM in which the oligosaccharides had been hydrolyzed by incubation with α-galactosidase. A series of studies from our laboratory support these results and demonstrated that the oligosaccharides raffinose and stachyose do not pose a nutritional concern and the use of α-galactosidase addition to enhance their digestibility would not be expected to produce a beneficial effect in chick performance (Slominski and Meng, unpublished). On the other hand, Refstie et al. (1999) assessed nutrient digestibilities of broilers fed different soybean products varying in the content of NSP and concluded that the lower nutrient digestibilities of regular SBM may be associated with antinutritive effects of the NSP fraction rather than the oligosaccharide fraction.

2.1.5. Peas

Peas contain 44% starch (Longstaff and McNab, 1987), and between 23 and 24% crude protein (DM basis, Fleury, 2004). The amino acid profile of peas is relatively well

balanced (Canibe and Eggum, 1997). Like many grain legumes, peas are deficient in methionine but contain relatively high levels of lysine (Perez-Maldonado et al., 1999), which complements the amino acid profile of cereals when used in poultry diets. Although the protein concentration in peas is lower than that in SBM or CM, the ME is generally higher (from 2725 to 3083 kcal/kg; Igbasan et al., 1997a). The good balance between crude protein and ME makes peas a valuable protein source for all types of poultry (Castell et al., 1996). The inclusion of raw peas in poultry diets is, however, restricted (Igbasan and Guenter, 1996; Cowieson et al., 2003), due to limited availability of some nutrients as well as the presence of antinutritive factors, including trypsin inhibitors, lectins, tannins, and phytic acid. However, it has been speculated that the antinutritive effects of these constituents appear negligible in Canadian peas (Castell et al., 1996; Fleury, 2004). Peas also contain from 4 to 6% oligosaccharides (Reichert and MacKenzie, 1982). Carre et al. (1995) reported high digestibilities of pea oligosaccharides in cockerels (>90%) and chicks (>70%). Addition of pea oligosaccharide extract (2.8-5.6%) to the diets of young broilers did not affect performance or digestibility of dietary nutrients (Trevino et al., 1990). Studies with pea protein concentrate indicated significant levels of endogenous α-galactosidase activity (Fleury, 2004) which may explain the lack of antinutritive effects of oligosaccharides in peas.

There is strong evidence that the digestibility of pea starch is lower than that of cereals (Longstaff and McNab, 1987). Pea starch has a higher amylose : amylopectin ratio compared with cereal starches. According to Daveby et al. (1998), amylose is relatively less digestible by nonruminants. Also, pea starch has a crystalline structure of

C type granule, which is more resistant to pancreatic amylase than A type typical of cereal starch granules (Daveby et al., 1998; Canibe and Bach Knudsen, 1997). Using microscopical evaluation, Wursch et al. (1986) demonstrated that the starch and protein in peas are encapsulated by cell wall polysaccharides which are responsible for the low or slow digestibility of starch. In this regards, examination of starch distribution in fractions of chicken excreta differing in particle size strongly suggested that starch and protein digestibilities were affected by the low accessibility of endogenous enzymes to nutrients present in the coarse particles (Carre et al., 1991). Significant improvements in starch and protein digestibility in broiler chicks were observed with fine versus coarse grinding of feed peas (Daveby et al., 1998).

2.1.6. Full-fat Oilseeds

Full-fat oilseeds such as canola and flax contain 41 to 43% oil and 20 to 25% protein (Lee et al., 1991) and therefore are valuable sources of energy and protein (Leeson et al., 1978; Shen et al., 1983; Salmon et al., 1988). In addition to providing considerable amounts of energy, the lipids of these seeds are an excellent source of α-linolenic acid (18:3ω3) with canola oil containing 8-12% and flax oil 48-58% (Ajuyah et al., 1991). It has been shown that 18:3ω3, and its desaturation products docosahexaenoic acid (22:6ω3) and eicosapentaenoic acid (20:5ω3) are important in human health, especially for those individuals with risk from chronic heart disease (Sim et al., 1991). Therefore, over the last few years there has been interest in feeding full-fat flax and canola seeds to poultry. Also, intact full-fat oilseeds are more resistant to oxidation than extracted and refined oils and might therefore be used as sources of dietary fat to

overcome the stability problems that might occur with the use of ordinary fats (Sim et al., 1991).

Although canola seed suffers from the same problems as CM, the content of antinutritive components in the seed is diluted due to its high oil content. As a result, considerable amounts of raw full-fat canola seed can be included in poultry diets. However, glucosinolates and other factors may lead to reduced performance when high inclusion rates (>15%) are used (Leeson et al., 1978; Summers et al., 1982; Shen et al., 1983). Inclusion of flaxseed in poultry diets has been reported to cause reduced energy utilization and depressed growth performance (Ajuyah et al., 1991; Lee et al., 1991; 1995). These effects seem to be due to the presence of various antinutritive factors such as mucilage, linatine, cyanogenic glycosides, trypsin inhibitors, and phytic acid (Madhusudhan et al., 1986; Bhatty, 1993). Flaxseed contains substantial amounts of water-soluble polysaccharide mucilage (Rodriguez et al., 2001), which is present in the hull of the seed. The viscous properties of mucilage are a major contributing factor to the antinutritive effects of flaxseed (Alzueta et al., 2003) as demonstrated using raw and demucilaged flaxseed in broiler feeding.

The ME contents of full-fat canola and flaxseeds have been documented to be lower than their corresponding meal-oil mixtures (Lee et al., 1991; 1995). The depressed ME values for the seeds may be due to a lower fat availability resulting from a physical barrier of cell walls to fat utilization from the whole seed. Heat and mechanical (e.g., flaking and extrusion) treatments of oil seeds have been proven beneficial (Leeson et al., 1978; Shen et al., 1983; Salmon et al., 1988) in poultry feeding probably due to a disruption of the cell wall structure.

2.2. NSP of Feedstuffs

2.2.1. NSP Content

The NSP content reported in the literature varies not only between different feedstuffs, but also within the same feedstuff due to differences in growing conditions, variety, analytical techniques or, as may be the case for SBM, the degree of hull removal. Typical NSP contents of common feedstuffs are summarized in Table 1.

2.2.2. NSP Structure and Composition

Nonstarch polysaccharides present in feedstuffs for poultry are a major component of cell walls and are closely associated with other minor non-carbohydrate components such as glycoproteins, phenolic acids, and lignin (Selvendran et al., 1987; Bacic et al., 1988). The carbohydrate fraction (i.e. NSP) of cell walls consists of cellulose microfibrils embedded in non-cellulosic polysaccharides. Cellulose constitutes a small proportion of cell walls of feedstuffs and is thought to be of little nutritional consequence. The majority of the carbohydrate fraction is derived from heteropolymers such as β-glucans and arabinoxylans present in most cereal grains and pectic polysaccharides present in most vegetable proteins. The presence of small amounts of protein and phenolic acids and their associations with the NSP are important because it is likely that they will influence the manner in which the NSP behave when ingested by animals. Water-solubility is an important property which determines the antinutritive effects of NSP in broiler diets (Annison, 1993). The solubilities of NSP are determined not only by their primary structure, but also by their association with other cell wall components (Smits and Annison, 1996). Most of the soluble NSP of cereal grains derive from the

TABLE 1. Nonstarch polysaccharide content of feedstuffs (g/kg)

Feedstuff	Content	Reference
Wheat	73-95	Cowan et al. (1996)
	97	Bach Knudsen (1997)
	106	Steenfeldt et al. (1998b)
	83-129	Austin et al. (1999)
	102	Danicke et al. (1999a)
	83-98	Slominski et al. (2000)
	98-117	Steenfeldt (2001)
	82-128	Pirgozliev et al. (2003)
Corn	69	Cowan et al. (1996)
	119	Bach Knudsen (1997)
	87	Danicke et al. (1999a)
	69	Slominski (2000)
	111	Chesson (2001)
Soybean meal	164-222	Irish and Balnave (1993)
	174	Cowan et al. (1996)
	217	Bach Knudsen (1997)
	145	Huisman et al. (1998)
Soybean meal (44% protein)	163	Danicke et al. (1999a)
Soybean meal (48% protein)	124	Danicke et al. (1999a)
	191	Slominski (2000)
Canola meal	180	Slominski and Campbell (1990)
Rapeseed meal	272	Cowan et al. (1996)
	220	Bach Knudsen (1997)
Peas	169	Cowan et al. (1996)
	180	Bach Knudsen (1997)
	142	Igbasan et al. (1997a)
	168	Danicke et al. (1999a)

thin-walled endosperm cells and reflects the composition of the endosperm wall (Steenfeldt et al., 1995).

The building blocks of the cell wall polysaccharides are the pentoses arabinose and xylose, the hexoses glucose, galactose and mannose, the uronic acids glucuronic and galacturonic acids, and the 6-deoxyhexoses rhamnose and fucose (Bacic et al., 1988; Theander et al., 1989). The main polysaccharides of feedstuff cell walls are cellulose, arabinoxylans, mixed linked β-glucans, xyloglucans, rhamnogalacturonans, arabinogalactans, etc. (Bacic et al., 1988; Theander et al., 1989). The main NSP structures, commonly found in feed ingredients of plant origin, are listed in Figure 1. In wheat and corn arabinoxylans predominate (Henry, 1987), although significant amounts of β-glucans and cellulose are also present (Steenfeldt et al., 1995). However, cellulose and pectic polysaccharides are the major cell wall constituents in vegetable proteins (Bacic et al., 1988, Bach Knudsen, 1997).

Cellulose is a linear homopolymer of β-1,4 glucose units. Glucan chains of cellulose are held together in an organized manner by inter and intramolecular hydrogen bonding which renders this carbohydrate insoluble, thus serving as the structural polysaccharide in plants.

β-Glucan is a linear polymer of glucose with β-1,4 and β-1,3 linkages (Theander et al., 1989). The presence of β-1,3 linkages differentiates β-glucans from cellulose and prevent close packing of the β-1,4 chains to give a more soluble and viscous polymer (Carre, 2002). The pattern of β-1,3 and β-1,4 linkages may affect the intermolecular association of β-glucan and its solubility. In this regard, approximately 85% of barley β-glucans consist of 2 to 3 β-1,4 bonds separating each β-1,3 linkage. The remaining β-

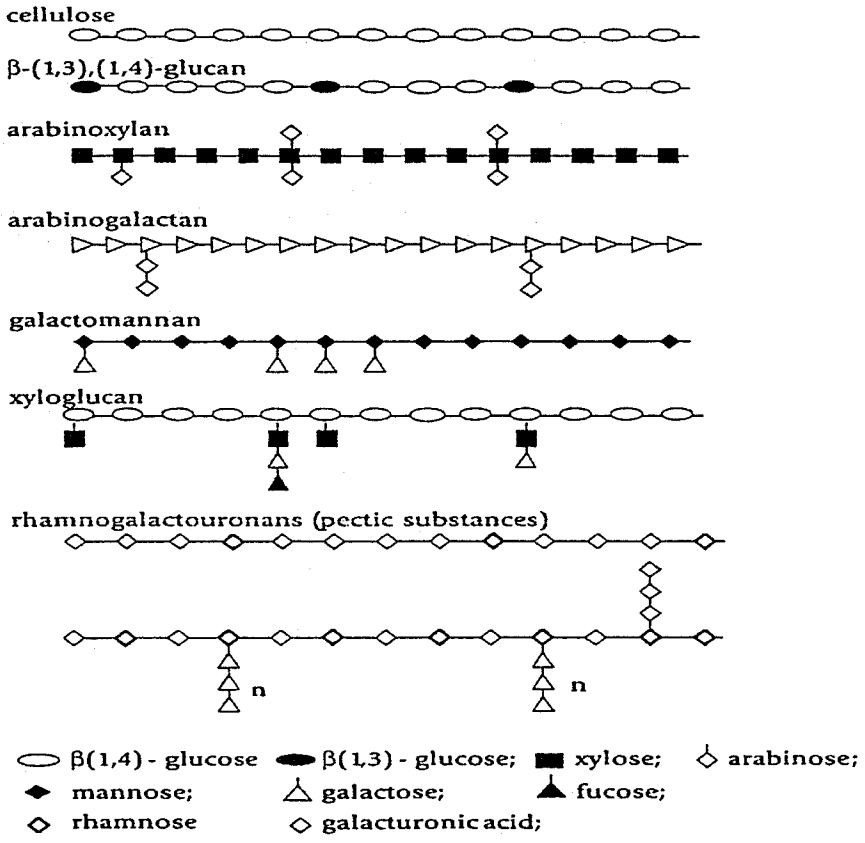

FIGURE 1. Polysaccharide structures commonly found in feed ingredients of plant origin (Smits and Annison, 1996).

glucans contain longer sequences of β-1,4 bonds again separated by a single β-1,3 connection (Classen and Bedford, 1991). Such structural arrangement has been suggested to give the barley β-glucan chains less opportunities to associate with insoluble cell wall fractions and consequently results in more water-soluble fractions (Graham et al., 1988).

The primary cell wall NSP of wheat and corn endosperms, within which starch and protein are located, are arabinoxylans (Henry, 1987). This polysaccharide is in a more complex and branched structure than cellulose and β-glucans and consists of a backbone of β-1,4-linked xylose residues with terminal 1,2 and 1,3 arabinose substitutions. Arabinoxylans differ between and even within grains with respect to the degree of arabinose substitution (Bedford, 1995). Close arabinose substitution on the xylose backbone reduces the ability for hydrogen bonding between carbohydrate chains and consequently results in fractions which are water-soluble and highly viscous. However, when the xylan backbone has sufficient zones without branching, the arabinoxylans are often linked with cellulose microfibrils through hydrogen bonds, resulting in water-insoluble fractions (Carre, 2002). In wheat, they were found to be bound to lignin and also protein present in the cell walls by various covalent linkages, with phenolic acids (i.e., ferulic acids) as intermediate compounds for example (Smits and Annison, 1996; Carre, 2002). Such structural characteristics of the arabinoxylans render them water-insoluble. In general, arabinoxylans found in the bran or outer aleurone layers of wheat have fewer arabinofuranosyl substitutions and are less water-soluble than those found in endosperm fractions (Henry, 1987). Wheat arabinoxylans have a great variation in water-solubility (Saulnier et al., 1995) and this may result from

the variation in frequency of arabinose substitutions or branching on the xylose backbones.

Even more complex polysaccharides are present in vegetable proteins. The pectic polysaccharides in SBM, CM, and peas are rhamnogalacturonans in which α-1,4 galacturonan chains are interrupted at intervals by insertion of α-1,2 rhamnose residues (Bacic et al., 1988). Other constituent sugars attached as side chains include galactose, arabinose, xylose, and less frequently fucose and glucuronic acid. Most of these sugars occur in short side chains, although galactose and arabinose are often found in multiple units (Bacic et al., 1988). In addition, neutral polysaccharides lacking the galacturonic acid backbone, such as arabinans, galactans, galactomannans, and arabinogalactans are present as separate polysaccharides or as long and highly complex branched side chains on the uronic acid backbone (Aspinall and Cottrell, 1971; Siddiqui and Wood, 1972; Daveby and Aman, 1993). Other polysaccharides in SBM, CM, and peas include cellulose, xylans, arabinoxylans and xyloglucans, which are predominantly found in the hull fraction.

In addition to the polysaccharides commonly found in vegetable proteins, flaxseed contains mucilage, a mixture of branched chain polysaccharides, in its hulls (3-9% of the DM of the seed; Mazza and Biliarderis, 1989). Flax mucilage consists of 2 types of polysaccharides, a neutral arabinoxylan and an acidic pectic-like polysaccharide containing rhamnose, galactose and galacturonic acid residues (Muralikirishna et al., 1987; Cui et al., 1994). The neutral arabinoxylan fraction contains a β-1.4-xylan backbone to which arabinose and galactose side chains are attached (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). Moreover, it was observed that the viscosity of flaxseed was high when compared with that of wheat (Carre, 2002). This may be related, in part, to the water-soluble arabinoxylan content which is much higher in flaxseed than in wheat (Saulnier et al., 1995; Rodriguez et al., 2001). This, however, may also be a result of differences in the distribution of branching points on the xylose backbones. Flax arabinoxylans perhaps have lesser lengths of xylose segments with no branching point which results in higher solubility and viscosity.

2.3. Mechanisms by Which Dietary NSP Affect Nutrient Utilization and Poultry Performance

Animals do not possess endogenous enzymes capable of cleaving and digesting cellulose, arabinoxylans, β-glucans or pectins. Much interest has focused on this fact and on ways of removing these polymers since they exert antinutritive effects and affect nutrient utilization by poultry. Early work has centered on viscous polysaccharides of cereal grains, such as rye and barley. Feeding diets rich in rye or barley, especially to young poultry, has long been known to depress growth rate and feed-to-gain ratio (Antoniou et al., 1980; Antoniou and Marquardt, 1980; White et al., 1983; Pettersson and Aman, 1988; Pettersson and Aman, 1989). Similar detrimental effects were also observed for cell wall polysaccharides present in wheat, although of a much smaller magnitude (Annison, 1992; Bedford and Classen, 1992; Choct and Annison, 1992a; Friesen et al., 1992; Marquardt et al., 1994; Steenfeldt et al., 1998a, b; Choct et al., 1999).

Although the exact antinutritive effects of NSP have not yet been established, two mechanisms have been proposed. The first mechanism relates to high water-solubility of

NSP present in the endosperm cell walls of cereal grains which when dissolved in the lumen increase the viscosity of the intestinal contents. Increased intestinal viscosity is known to impede nutrient digestion and absorption (White et al., 1981; 1983; Annison and Choct, 1991; Bedford and Classen, 1992; Choct and Annison, 1992a, b). The second mechanism is that the polysaccharides encapsulate starch, fat and protein within the endosperm cells and thus inhibit the access of endogenous digestive enzymes to these nutrients (Hesselman and Aman, 1986; Pettersson and Aman, 1989). Generally, the antinutritive effects of NSP are explained by increasing digesta viscosity on one hand and by the nutrient encapsulating effect on the other hand (Bedford, 1996; Simon, 1998; Bedford, 2002). In addition to direct effects on nutrient digestion and absorption, modifications of quantity and composition of the intestinal microflora may also be involved in the antinutritive effects of NSP (Simon, 1998; Bedford and Apajalahti, 2001).

2.3.1. Inducing Intestinal Viscosity

It is well accepted that the gel-forming capacity of the NSP of barley and rye, with the resultant increase in viscosity of the small intestinal contents, is the major mechanism by which these grains reduce the performance of poultry (Antoniou and Marquardt, 1982; White et al., 1983). After ingestion, β-glucans and arabinoxylans become soluble and increase digesta viscosity (Classen and Bedford, 1991). This has been examined in great detail in a dose response study using 4 levels of rye substituting for wheat (Bedford and Classen, 1992). The results demonstrated that the depressed growth rate and feed-to-gain ratio were closely correlated with the increase in digesta viscosity. This relationship was found to be true when all rye-containing diets were

eliminated from data analysis, suggesting that intestinal viscosity may be a growth limiting factor even for wheat-based diets.

The water-soluble arabinoxylans (which are more concentrated in rye than in wheat) and β-glucans (most concentrated in barley) have been identified as being the fractions responsible for the elevation in intestinal viscosity and thus the depression in nutrient utilization from rye- and barley-based diets (Antoniou and Marquardt, 1982; White et al., 1983). In the case of wheat, Annison (1990) reported that the AME of the wheats (13 wheat samples obtained from seed suppliers across Australia) negatively correlated with the water-soluble arabinoxylans ($r=-0.86$; $P<0.001$) and total water-soluble NSP levels ($r=-0.91$; $P<0.001$). This suggests that one factor which determines the nutritive value of wheat is the level of soluble arabinoxylans in the grain. Isolation of these fractions and re-feeding to poultry resulted in a significant increase in intestinal viscosity and concomitant depression in weight gain, feed-to-gain ratio, and AME of the diet, the responses seen when the intact grain was fed (Choct and Annison, 1990, 1992b). This confirms that the detrimental to growth fraction in wheat is the water-soluble arabinoxylans. It was further demonstrated that depolymerization of the isolated arabinoxylans decreased the viscosity of ileal digesta of the birds and as a result no significant reduction in the AME content of the diets was observed (Choct and Annison, 1992b).

While the exact effects of viscosity have not been established, it appears that an increased intestinal viscosity reduces the diffusion rate of substrates, digestive enzymes and end-products in the gut lumen (Fengler and Marquardt, 1988) which is detrimental for rapid nutrient digestion. In addition, Edwards et al. (1988) suggested that viscous

polysaccharides reduce absorption due to a decrease in the convective transport of nutrients. The result of such effects of NSP would obviously be a reduced nutrient assimilation. Consequently, the depression in starch, protein and fat digestibilities is frequently correlated with digesta viscosity (White et al., 1981; 1983; Annison and Choct, 1991; Bedford and Classen, 1992). Also, the larger the molecular size of the nutrient, the greater the extent to which its rate of diffusion is reduced (Bedford, 1995). These observations contribute to the finding that fat digestion suffers the most pronounced impairment due to high digesta viscosity (Campbell et al., 1983; Ward and Marquardt, 1983; Choct and Annison, 1992b), probably owing to the large size of the fat micelle relative to more simple products of digestion in the intestine (Campbell et al., 1983). Furthermore, an increase in intestinal viscosity is more detrimental to the digestion and absorption of dietary fats containing high proportions of saturated fatty acids. Antoniou et al. (1980) and Danicke et al. (1997, 1999b, 2000) observed a greater depression in performance and fat digestibility in birds fed rye-based diets when beef tallow rather than soya oil was added to the diet.

It is well known that beef tallow is characterized by low digestibility, particularly in young birds (Ketels and DeGroote, 1989). Ward and Marquardt (1983) attributed such poor digestibility of tallow to the degree of saturation of its fatty acids. Beef tallow contains mainly long chain saturated fatty acids (LCSFA), such as palmitic and stearic acids, and unsaturated oleic acid (NRC, 1994). Danicke (2001) suggested that the LCSFA in beef tallow are nonpolar and thus rely on an adequate presence of bile salts for efficient emulsification and micelle formation, which are essential for fat digestion and absorption. Contrary to beef tallow, vegetable oils, such as soya oil and canola oil, are

primarily composed of long-chain unsaturated oleic, linoleic, and linolenic acids (NRC, 1994), which can be easily absorbed even in the absence of bile salts (Garret and Young, 1975). Despite the need for bile salts to digest tallow, birds younger than 3 wk have been observed to produce inadequate secretions of bile acids, particularly when tallow was provided as dietary fat (Krogdahl, 1985). Support for this was given by Polin et al. (1980) and Fengler et al. (1988), who showed that feeding exogenous bile salts to chicks increased their ability to digest tallow. In addition, studies by Kritchevsky and Story (1974) and Kritchevsky (1978) have demonstrated that fibres from different feedstuffs are able to bind bile acids in vitro. Consequently, such binding could contribute to an increase of their excretion by birds. The viscous NSP of feedstuffs might exert a similar effect on bile acids and increase bile acid excretions (Langhout et al., 1997). This might further exacerbate the deficiency of bile salts in the young broilers. In addition, lipase activity was reported to be low in the very young chick (Nir et al., 1993) and lipase addition to a tallow-containing diet was shown to improve fat digestibility (Polin et al., 1980).

Smulikowska (1998) suggested that an increased intestinal viscosity caused by ingestion of water-soluble NSP of viscous cereal grains, such as rye, might lead to reduced gut motility, which in turn may decrease the rate of diffusion and the convective transportation of emulsion droplets, fatty acids, mixed micelles, bile salts and lipase within the small intestine. Such a situation would be particularly detrimental for the digestion of LCSFA, as they rely more on vigorous digesta mixing for optimal emulsification. In addition, Krogdahl (1985) suggested that both palmitic and stearic fatty acids are nonpolar and cannot spontaneously form mixed micelles, but can be solubilized

into micelles formed from unsaturated fatty acids and conjugated bile salts. Therefore, such dependence of digestion and absorption of LCSFA upon the presence of bile salts, unsaturated fatty acids, and formed micelles would explain why tallow digestion is more sensitive to small increases in intestinal viscosity.

A greater depression in fat digestibility of tallow than vegetable oils is often observed for broilers fed rye-based diets which often give rise to high intestinal viscosity. When compared with rye, wheat contains a lower level of arabinoxylans and thus produces lower viscosity (Henry, 1987). However, Preston et al. (2001) demonstrated a significant lower fat digestibility when a 70% wheat-based diet was supplemented with beef tallow rather than the vegetable oils. Furthermore, Pasquier et al. (1996) observed reduced fat emulsification and hydrolysis with every increment in medium viscosity over a range from 0 to 20 mPa s. These results suggest that the negative effect of viscous NSP on fat digestion and absorption may not be confined to diets that induce high intestinal viscosity.

2.3.2. Encapsulating Nutrients

The association of water-soluble NSP and the resultant high intestinal viscosity with reduced nutrient utilization in rye, barley, and even wheat-based diets is without question. It seems rather unlikely, however, that intestinal viscosity alone is responsible for all the antinutritive effects of NSP. The high viscosity rye or wheat also contains a higher concentration of water-insoluble NSP which may act as a physical barrier preventing or slowing down the access of endogenous enzymes to starch, protein, and fat encapsulated by the cell wall structure. This, in turn, reduces the utilization of these nutrients (Hesselman and Aman, 1986; Pettersson and Aman, 1988; 1989). Such cell wall

encapsulation effect relates to the fact that the feed manufacturing processes, such as grinding and pelleting, does not break open all cells of the endosperm. With ground and pelleted feed, the gizzard also fails to develop fully, and as a result many complete, intact particles of feed enter the gut (Svihus et al., 1997). Consequently, the contents of some cells may escape digestion.

The microscopy study by Bedford and Autio (1996) demonstrated that there was indeed a considerable amount of starch surrounded by intact cell walls in the intestinal digesta of broilers fed wheat-based diets. This confirms that the physical entrapment of starch and protein by cell wall polysaccharides may be another important factor by which NSP exert their antinutritive properties (Theander et al., 1989; Bedford and Autio, 1996; Wiseman et al., 2000).

The cell wall polysaccharides present in corn, SBM, CM, peas and oilseeds are of lower solubility compared with those of rye or wheat, and thus generally do not pose the viscosity problems. However, these NSP may cause poor nutrient utilization by limiting or slowing down the access of endogenous enzymes to the nutrients (i. e., protein, starch, or fat) encapsulated by the cell wall structure.

There is evidence suggesting that the digestibility of corn starch is not as high as previously thought. Although starch digestibility when determined by excreta collection is generally regarded as being in excess of 98%, recent studies have shown that up to the end of the small intestine of broilers between 4 and 21 days of age, starch digestibility could be as low as 82%, with no evidence of an increase as the birds get older (Noy and Sklan, 1995). This evidence suggests that a significant proportion of corn starch may pass undigested to the hindgut, where it will be fermented or utilized by the microflora.

However, utilization of nutrients through microbial conversion of digestible carbohydrate, such as starch, to short chain fatty acids (SCFA) is not as efficient as direct absorption of glucose following enzymatic digestion (Carre et al., 1995). Similar to what was found for broilers fed wheat-based diets using microscopic examination of the ileal digesta (Bedford and Autio, 1996), large corn endosperm particles were also found to remain undigested within the chick ileum. This confirms that, similar to wheat starch, corn starch digestion may be affected by the physical barrier created by the aleurone layer and starchy endosperm cell walls. This would evidently limit the bird's amylases in accessing and fully digesting the starch component enclosed within the cells (Theander et al., 1989; Slominski et al., 1993; Bedford, 2002). It has been suggested that not only starch, but also a considerable amount of corn protein could be shielded by cell walls and thus escape digestion (Pack et al. 1998).

Feed peas contain high concentration (45%) of starch in the cotyledons (Castell et al., 1996). Using microscopical examination, Wursch et al. (1986) demonstrated that pea starch, together with associated protein, is located in parenchyma cell walls which may limit starch digestibility. Slowed or impeded starch digestion due to the encapsulation effect of pea NSP has also been suggested by other studies (Longstaff and McNab, 1987; Daveby et al., 1998).

In the cotyledons of full-fat canola and flaxseeds, oil is located in numerous thick-walled cells (Sosulski and Sosulski, 1993). The polysaccharides of the cell walls can largely be disrupted by mechanical processing. However, due to the small size of canola and flaxseeds, grinding may not effectively or completely disrupt the cell walls of these seeds. Therefore, the utilization of oil from these oilseeds may be limited.

2.3.3. Interaction with Microflora

The primary cause of the reduced rate of nutrient digestion resulting from NSP seems to be related to their viscous properties and/or nutrient encapsulating effect. However, it is important to emphasize that the microflora of the gastrointestinal tract may be partially responsible for the detrimental effects. Recent evidence suggests that the reduced rate of nutrient digestion is much more pronounced in the presence of intestinal microflora than in their absence. For example, Choct et al. (1992) reported that the addition of wheat arabinoxylans to broiler diets significantly depressed nutrient digestibility and performance in intact birds, whereas no such depressions were noted in caecectomized chickens. Other researchers have reported that dietary supplementation with antibiotics partially improves the nutritive value of rye (Misir and Marquardt, 1978), suggesting that the antinutritive effect of NSP may be mediated by the gut microflora. The positive effect of antibiotics appears to be due to the elimination of fermentative microorganisms from the small intestine. In another study, Choct et al. (1996) demonstrated that addition of soluble NSP to broiler chicken diets significantly elevated microbial fermentation in the small intestine. Subsequent in vivo depolymerization of the soluble NSP almost totally overcame this problem. It might therefore be concluded that the negative effects of NSP are due, at least partially, to some interactions with the gut microflora.

As indicated earlier, the viscous intestinal environment created by the water-soluble and viscous NSP impedes rapid digestion of nutrients and increases the average retention time of chime in the gastrointestinal tract (Salih et al., 1991). Consequently, more unabsorbed material reaches the ileum and may promote the proliferation of

detrimental microflora (Salih et al., 1991). This provides the microflora with more substrates and more time to migrate and to colonize the upper segments of the small intestine (Smits and Annison, 1996). Since the microflora is able to utilize starch and protein, it would compete effectively with the host for nutrients (Bedford, 1995), resulting in poor energy utilization efficiency.

In addition, some intestinal bacteria produce bile acid degrading enzymes and any increase in bacterial activity may cause an increase in the deconjugation of bile acids, which may further reduce the fat digesting capabilities of the bird (Feighner and Dashkevicz, 1988; Christle et al., 1997; Smits et al., 1998). Since bile acids are also thought to stabilize pancreatic proteases in the intestinal lumen, protein digestion could also be compromised. Many of these problems may be alleviated by inclusion of bile acids or antibiotics (Campbell et al., 1983).

2.4. NSP Degrading Enzymes

2.4.1 Possible Mode of Action of Carbohydrase Enzymes

On account of the antinutritive properties of the cell wall NSP present in feedstuffs, a great deal of research has focused on the means of alleviating their detrimental effects. The use of the appropriate NSP degrading carbohydrase enzymes (e.g. xylanase, glucanase) was shown in many studies to significantly improve the nutritive value of cereal grains, such as barley, rye, or wheat (Antoniou et al., 1980; Antoniou and Marquardt, 1980; White et al., 1983; Annison, 1992; Bedford and Classen, 1992; Choct and Annison, 1992a; Friesen et al., 1992; Marquardt et al., 1994; Steenfeldt et al., 1998a,

b; Choct et al., 1999). Therefore, NSP degrading enzymes are currently being widely used as feed additives in cereal based diets, especially for young poultry.

Corresponding to the antinutritive effects of NSP, the mode of action of carbohydrase enzymes is related to partial hydrolysis of soluble and insoluble NSP in the upper digestive tract, leading to a decrease in digesta viscosity and elimination of nutrient encapsulating effect of cell walls. In addition to direct effects on nutrient digestion and absorption, quantitative and qualitative modifications of the intestinal microflora seem to be involved in the overall responses to the NSP degrading enzymes (Simon, 1998; Bedford and Apajalahti, 2001).

2.4.2. Intestinal Viscosity Reduction

Numerous studies indicate that partial hydrolysis of NSP in the gut by carbohydrase enzymes is directly reflected by changes in the viscosity of intestinal digesta. For instance, White et al. (1981) and Bedford et al. (1991) demonstrated that supplementation with enzymes capable of degrading β-glucans and arabinoxylans (i.e. glucanases, xylanases) increased the nutritive value of barley and rye in poultry diets, which coincided with a significant decrease in the digesta viscosity. Also, improvements in growth performance and feed efficiency in broilers fed wheat-based diets supplemented with various xylanase preparations have been frequently found to be closely correlated with a reduction in viscosity of intestinal contents (Annison, 1992; Bedford and Classen, 1992; Choct and Annison, 1992a; Friesen et al., 1992; Marquardt et al., 1994; Choct et al., 1995; Steenfeldt et al., 1998a, b; Choct et al., 1999).

It has to be emphasized that the improvements in the nutritive value of cereal grains following enzyme addition can not be attributable to the release of constituent

sugars from targeted polysaccharides and their subsequent utilization as the energy source. Although monosaccharides are released following some enzyme addition, it is unlikely that sufficient amounts would be produced from any type of the cell wall polysaccharides considering a relatively short residence time in the foregut (Isshiki et al., 1989). In fact, some sugars (e.g. arabinose and xylose) are so poorly absorbed and/or utilized (Longstaff et al., 1988; Schutte et al, 1991) that it may be more advantageous to allow them to pass into the hindgut as oligosaccharides. The energy yield from the fermentation of these oligosaccharides by the hindgut flora could be greater than that available from absorption of the equivalent monosaccharides in the foregut (Chesson, 1993). Rather, the beneficial effects of enzyme addition would derive from the release of constraints on digestion due to the presence of NSP. It would appear that carbohydrase enzymes hydrolyze the cell wall polysaccharides to low-molecular weight components and thus decrease digesta viscosity leading to improved nutrient digestion and absorption (Edwards et al., 1988; Pasquier et al., 1996).

Fat digestion requires vigorous peristalsis to ensure optimum emulsification (Pasquier et al., 1996). Hence, it is not surprising that the effects of viscosity reduction on fat digestibility are significant. Numerous studies have demonstrated that the reduction of viscosity through the use of enzymes increases the digestibility of fat more so than any other nutrients (Choct and Annison, 1992b; Silva and Smithard, 1997; Steenfeldt et al., 1998a, b; Danicke et al., 1999b, 2000). As a result, most of the enzyme-related studies, in which fat digestibility had been determined, have suggested that improvements in fat digestibility are responsible for much of the improvements in growth performance (Campbell et al., 1983; Choct et al., 1992; Danicke et al., 1997; 1999b).

Viscosity reduction is known to have a much greater effect on digestion of saturated fats such as beef tallow than on utilization of unsaturated fats such as vegetable oils. This suggests that there is an interaction between dietary fat type and carbohydrase addition and such interaction may be more pronounced for diets that induce high digesta viscosity, such as rye-based diets. In this regard, a number of studies have demonstrated that enzyme supplementation to rye-based broiler diets resulted in more pronounced improvements in nutrient digestibility and performance when beef tallow rather than vegetable oil was used as a fat source (Antoniou et al., 1980; Smulikowska and Mieczkowska, 1996; Danicke et al., 1997, 1999b, 2000; Langhout et al., 1997). As indicated earlier, a relatively lower intestinal viscosity is often observed for wheat- than rye-based diets. However, using wheat-based (70%) diets, Preston et al. (2001) compared the effects of enzyme addition to diets containing either vegetable oil (soya, 10%) or animal fat (beef tallow, 10%). Although bird performance and fat digestibility were only slightly enhanced by enzyme supplementation to the soya oil-based diet, the improvement was significantly greater with the diet containing beef tallow. This suggests that the interaction between fat type and enzyme effect may apply to wheat-based broiler diets as well. However, in many studies such fat type and enzyme interactions were evaluated using high inclusion rates of fat (e.g. 10%) and highly viscous semisynthetic diets (e.g. rye-based). Whether or not such interaction exists in practical wheat-based broiler diets with a moderate wheat inclusion rate (e.g. 60%) and fat inclusion rate (e.g. 5%) is not clear.

2.4.3. Elimination of Nutrient Encapsulating Effect of Cell Walls

Nonstarch polysaccharide-degrading enzymes, such as xylanases, glucanases, and cellulases, have been widely used in viscous cereals based diets for young poultry. Their benefits have been credited to the partial breakdown of water-soluble and viscous NSP of rye, barley or wheat and the resultant decrease in digesta viscosity (Bedford and Classen, 1992). However, reduction in intestinal viscosity probably does not explain all benefits resulting from enzyme use. In earlier studies, Pettersson and Aman (1988; 1989) found that supplementation of rye-based diets with xylanases improved weight gain of broilers without a significant decrease in digesta viscosity. These workers suggested that although the xylanase did destroy the viscous property of the soluble arabinoxylan fraction, it simultaneously degraded the otherwise insoluble polysaccharides of the cell walls leading to the release of additional polymers, which maintained the overall high viscosity of the foregut contents. Hence, these researchers concluded that disruption of the intact walls and release of nutrients was the major factor responsible for any improvement in nutritive value ascribed to exogenous enzymes. This was also suggested by Hesselman and Aman (1986) in relation to β-glucanase treatment of barley.

In the case of wheat, recent evidence suggests that the positive responses from enzyme supplementation are not always associated with a decrease in digesta viscosity (Veldman and Vahl, 1994; Dusel et al., 1998; Slominski et al., 2000; McCracken and Miller, 2002). It is noteworthy that such responses from enzyme supplementation generally occurred for wheat of relatively low viscosity. In this regard, the decrease in intestinal viscosity seems to be dependent on the intrinsic viscosity of the cereal. Using different varieties of wheat alone and in combination to achieve a range of viscosity

values from 1.5 to 3 cp, Barrier-Guillot et al. (1995) demonstrated a greater decrease of jejunal viscosity after xylanase supplementation with high-viscosity wheat compared with low-viscosity samples (Table 2). It is of interest to note that a decrease in jejunal viscosity was not observed for broilers fed wheat variety Soissons (the variety with relatively lower jejunal viscosity). However, following enzyme supplementation, the improved AME_n value was similar across wheat varieties irrespective of the viscosity value. Similarly, Slominski et al. (2000) evaluated four wheat samples of different quality with and without enzyme supplementation utilizing a 2-wk feeding trial with broiler chickens (Table 3). A more pronounced improvement in growth performance and AME following enzyme supplementation was observed for low-quality wheat. However, the reduction in digesta viscosity was not apparent for the low-quality wheat which actually had a relatively low digesta viscosity for the no-enzyme treatment. These observations further suggest that other factors may be of importance in situations when digesta viscosity is low.

Elimination of nutrient encapsulating effect of cell wall polysaccharides has been suggested as another important mechanism by which carbohydrase enzymes exert their beneficial effects. This is supported by Bedford and Autio (1996), who demonstrated that the jejunal contents of birds fed xylanase-supplemented wheat-based diets contained less undamaged endosperm cells than those of control birds, indicating that cell walls were in fact being degraded by exogenous enzymes. This would explain the more rapid and complete digestion of nutrients and the observed improvements in performance and AME content with enzyme supplementation.

TABLE 2. Effect of xylanase supplementation on the feeding value of different wheat varieties for broilers (Barrier-Guillot et al., 1995)

Cereal	Corn	Wheats							
		Soissons		Mixte		Thesee		Futur	
Viscosity		1.52		1.71		1.90		2.99	
Xylanase		–	+	–	+	–	+	–	+
FCR (15-28d)	1.56	1.59	1.55	1.63	1.55	1.64	1.56	1.62	1.56
Jejunal viscosity (cP)	2.7^{c}	3.2^{c}	3.1^{c}	4.1^{b}	3.2^{c}	4.5^{b}	3.2^{c}	6.6^{a}	3.1^{c}
AME_n (kcal/kg)	3390^{a}	3130^{c}	3280^{b}	3130^{c}	3290^{b}	3120^{c}	3240^{b}	3140^{c}	3250^{b}

TABLE 3. Effect of enzyme supplementation on broiler chicken performance (4-18 d) when fed wheat of different quality and composition (Slominski et al., 2000)

Type of wheat	Enzyme	Weight gain (g/bird)	Feed to gain ratio	AME MJ kg^{-1}	Viscosity CPs
Low starch, high protein	–	421 ± 13[1]	1.44 ± 0.02	12.59 ± 0.05	3.9 ± 0.9
	+	434 ± 13	1.42 ± 0.02	12.87 ± 0.14	2.6 ± 0.6
High starch, low protein	–	437 ± 14	1.39 ± 0.02	12.91 ± 0.24	3.0 ± 0.7
	+	431 ± 21	1.37 ± 0.03	13.07 ± 0.17	2.7 ± 0.4
Good quality	–	430 ± 13	1.42 ± 0.03	13.17 ± 0.31	3.2 ± 0.6
	+	428 ± 8	1.41 ± 0.02	13.19 ± 0.25	2.6 ± 0.3
Low quality	–	409 ± 12	1.43 ± 0.02	12.45 ± 0.26	2.6 ± 0.5
	+	427 ± 18	1.37 ± 0.03	12.91 ± 0.32	2.7 ± 0.3
Source		Probability			
Wheat type		*	**	**	*
Enzyme		NS	**	*	**
Wheat type × enzyme		NS	*	NS	*

[1] **Mean** ± SD; * P ≤0.05; ** P ≤0.01

Similar mode of action may apply to corn. As suggested earlier, there is a considerable amount of starch in corn encapsulated by the cell wall structure and many of these cells pass through the digestive tract without being exposed to endogenous enzymes. Therefore, there is an opportunity for the appropriate carbohydrase enzyme to be used to improve starch utilization by the bird.

Application of cell wall degrading enzymes has been proven beneficial in facilitating the extraction of canola oil in the aqueous extraction process (Sosulski and Sosulski, 1993). Increased degradation of water-soluble and water-insoluble NSP by the action of carbohydrase enzymes and the resultant improvement in NSP digestibility were noted for SBM- (Marsman et al., 1997; Kocher et al., 2002), CM- (Slominski and Campbell, 1990; Kocher et al., 2000) or peas- (Daveby et al., 1998) based diets for broilers.

It is postulated that a single enzyme should be effective if the beneficial effects of enzymes are due to reduced viscosity (Chesson, 1993). The reason is that viscosity is partially a function of chain length and so it is only necessary to break the chain in a few sites to reduce or destroy its viscosity-inducing capacity. However, if the beneficial effects of added enzymes are due to disruption of intact cell walls and release of entrapped nutrients, enzymes capable of hydrolyzing a wide array of complex linkages would be necessary (Slominski et al., 1993). These enzymes would be required in high quantity and to work in a coordinated way in order to digest cell walls to any significant extent in a relatively short period of time (Isshiki et al., 1989; Chesson, 1993; Slominski et al., 1993; Bedford, 1995). Earlier data from our laboratory have shown increased digestibility of nonstarch polysaccharides following supplementation of a poultry diet

with a multiactivity system of cell wall degrading enzymes (Slominski and Campbell, 1990).

2.4.4. Modification of the Intestinal Microflora with Enzyme Supplementation

It is frequently claimed that modifications of the intestinal microflora are involved in the effects of various carbohydrase enzymes. It has been proposed that the exogenous cell wall-degrading enzymes have two types of activity: removal and provision of substrates for microbial fermentation (Bedford, 2000). The former is more likely to be an effect seen in the small intestine and the latter in both lower small and large intestine.

Enzymatic depolymerization of viscous polymers and structural polysaccharides removes the constraints on nutrient digestion and absorption and increases the rate of starch and protein utilization from the small intestine. Such effects lead to less material being available for fermentative organisms to proliferate especially in the distal small intestine. Vahjen et al. (1998) studied the influence of xylanse supplementation on the development of selected bacteria in the intestinal tract of broilers fed wheat-based diets. Enzyme addition resulted in significantly lower counts of presumptive enterobacteria and gram-positive cocci in luminal and mucosa samples in the first 3 wks. They concluded that the less viscous intestinal environment resulting from the xylanase addition slowed proliferation of gram-positive cocci and presumptive enterobacteria.

Exogenous carbohydrases partially depolymerize NSP to produce smaller polymers, oligomers, or free sugars. For example, xylanase and cellulases ultimately produce xylose and glucose, respectively, and oligomers of various chain length and degrees of substitution (Austin et al., 1999). The small intestinal concentration of NSP of relatively lower molecular weight has been found to increase almost twofold following

addition of high levels of a xylanase enzyme (Bedford and Classen, 1992). More recently, Apajalahti and Bedford (1999) demonstrated that the addition of xylanase increased the concentration of xylo-oligomers dramatically and it was more pronounced for those being less than 10 xylose sugars long. Such oligomers are not being absorbed by the host, but enter the caeca and are broken down by the bacterial population (Danicke et al., 1999c). As a result, some reduction in the number of starch and protein users (Vahjen et al., 1998) and an increase in xylose users following xylanase addition was observed. (Apajalahti and Bedford, 1999).

Enzyme effect on microbial populations has been further examined by Choct et al. (1996). These authors demonstrated that feeding a sorghum-based diet supplemented with 3% of isolated water-soluble wheat arabinoxylan markedly depressed growth and feed efficiency and increased volatile fatty acid (VFA) production in the ileum (indicating an increased ileal microbial activity). Addition of xylanase to the diet restored the performance of the birds and reduced ileal VFA concentrations and significantly increased VFA concentration in the caeca. More detailed analysis of the profiles of the VFA revealed that the molar ratios were altered in favor of increased propionic acid, which in earlier research was identified as being harmful to *Salmonella* and other putrefactive bacteria (Hume et al., 1996). In another study (Apajalahti and Bedford, 1999), increments in caecal *Bifidobacteria* (propionic acid producer) counts following xylanase supplementation were observed. As a result, the increase in *Bifidobacteria* population relative to *Salmonella, Campylobacter and Clostridium* was observed. The latter (*Clostridium perfringens*) is a causative agent of necrotic enteritis, a serious disease in poultry (Truscott and Al-Sheikhly, 1977; Al-Sheikhly and Al-Saieg, 1980). The VFA

produced by such propionic acid producer may be of benefit not only in controlling the growth of pathogenic bacteria, but also in providing some energy for the bird.

2.5. The Effect of Carbohydrase Supplements on Poultry Performance and Nutrient Utilization

The potential for using enzymes in poultry diets to improve the nutritive value of cereal grains has been recognized for decades (Jensen et al., 1957). Among cereal grains, rye and barley have been proven to benefit from xylanase and β-glucanase supplementation and the practical application of these enzymes has been well established. However, the literature data suggest that the effect of enzyme supplementation is not as pronounced and consistent in wheat-based diets as in rye-based diets. This is due to a much lower concentration of soluble NSP in wheat compared with rye (Bedford and Classen, 1992). Corn contains even lower soluble NSP contents (Chesson, 2001) and is often referred to as a low-viscosity grain. Therefore, enzyme addition to corn-based diets often results in smaller, if any, beneficial responses. As an example, Marquardt et al. (1994) demonstrated that although enzyme supplementation improved the weight gain of broilers fed a wheat-based diet, no effect on corn-fed chicks was observed.

The successful use of enzymes in rye-, barley-, and, to some extent, wheat-based diets has stimulated interest in the application of enzymes to target the vegetable protein components of poultry feeds. A few studies reported to date have shown that this may be more challenging probably due to the more complex structures of the cell wall polysaccharides in vegetable proteins and the inability of currently available feed

enzymes to depolymerize these polysaccharides to any significant extent and improve performance within the short feed retention time in the gut.

2.5.1. Enzymes in Wheat-based Diets

A number of studies have shown that enzyme preparations containing xylanase activity are capable of improving the nutritive value of wheat-based diets (Pettersson et al., 1990; Annison, 1992; Bedford and Classen, 1992; Veldman and Vahl, 1994; Barrier-Guillot et al., 1995; Choct et al., 1995; Dusel et al., 1998; Steenfeldt et al., 1998a, b; Choct et al., 1999; Slominski et al., 2000; McCracken and Miller, 2002). Generally, the inclusion levels of wheat in the experimental diets used in most studies were in the range from 60 to 85%. In an experiment by Friesen et al. (1992) improvements in weight gain and feed-to-gain ratio of broiler chickens fed diets containing 70% of wheat were found to be 2.9% and 9.2%, respectively. Steenfeldt et al. (1998a) reported that enzyme supplementation to diets containing high amounts of wheat (81-84.5%) improved weight gain and feed conversion efficiency by 6% and 7%, respectively. However, when evaluating the enzyme addition to diets containing 63% and 72% of wheat, only the diet with 72% wheat inclusion rate showed an improvement in feed efficiency. Veldman and Vahl (1994) also found that the beneficial effects of xylanase addition to a wheat-based broiler diet were dependent on wheat content. It would appear that there is an interaction between wheat inclusion level and enzyme supplementation and the response from enzyme addition is more pronounced for high wheat-containing diets (Steenfeldt et al., 1998a; Preston, et al., 2001).

Enzyme addition to wheat-based diets generally improves chick performance by increasing AME and nutrient digestibility values. The review of the literature data

suggests that xylanase addition increases the AME of wheat with the degree of response being variable. Annison (1992) reported that various commercial enzyme preparations increased the AME of Australian wheat (80%) by 7.2-10.2%. Marquardt et al. (1994) demonstrated a significant improvement in AME (4%) of a crude xylanase supplemented diet based on Canadian wheat (70%). However, AME was unchanged by the addition of commercial enzymes to Canadian wheat (64%)-based diets in another study (Leeson et al., 2000). Choct et al. (1995) found that supplementation of a commercial xylanase preparation improved the AME value of the low ME wheat (AME = 12.02 MJ/kg DM) by 24.3%, but resulted in no improvement in AME of the normal ME wheat (AME = 14.52 MJ/kg DM). In addition, Hickling (1994) reported that a commercial preparation containing several different enzyme activities, including xylanases, reduced the variability in AME_n of 5 commercial Canadian wheat samples and created a sample set with a similar variability to the TME_n results (determined without enzyme addition). Similarly, the difference in AME values between the highest and the lowest of 9 cultivars of Canadian wheat was substantially reduced (from 10% to 4%) by the addition of enzyme (Scott et al., 1998).

Klis et al. (1995) evaluated the effects of a xylanase supplement on apparent fat digestibility in broilers fed diets containing 13 different wheat cultivars. On average, fat digestibility improved from 75.8 to 82.8%. Similar results were reported by Steenfeldt et al. (1998b), who demonstrated a significant improvement in apparent ileal and total tract fat digestibility in broilers fed enzyme-supplemented wheat-based diets.

Enzyme supplementation may also affect dietary protein utilization. The improvement in apparent protein digestibility in wheat-based diets with enzyme

supplementation has been reported (Friesen et al., 1992; Steenfeldt et al., 1998b). An increase in ileal and total tract amino acid digestibilities with enzyme addition has also been documented for Australian wheat (Hew et al., 1998).

Annison (1992) found a significant increase in the ileal digestibility coefficients of starch varying from 0.88 for control birds to 0.96-0.98 for birds fed enzyme-supplemented wheat-based diets. However, Steenfeldt et al. (1998b) observed only marginal effects of carbohydrase enzyme addition on apparent digestibility of starch in wheat-based diets, with the control treatment showing a very high starch digestibility. Starch digestibility was markedly improved by enzyme supplementation in the low ME Australian wheat, although the effects were not significant when feeding the normal ME wheat (Choct et al., 1995). Furthermore, Choct et al. (1999) demonstrated that, in addition to the improved starch digestibility, xylanase addition to the low-ME Australian wheat diet reduced the between bird variability in starch digestibility from 9% in control birds to 1.5% in enzyme-supplemented birds. This may be of great importance to the boiler industry since enzyme supplementation may lead to a more consistent and uniform performance.

Apart from improvements in apparent digestibility of starch, fat and protein, it has been shown that enzymatic degradation of NSP results in their improved utilization (Pettersson and Aman, 1989; Annison, 1992; Choct et al., 1995). In the study by Pettersson and Aman (1989), the apparent digestibility of NSP in the enzyme-supplemented wheat-based diets increased by 13% compared to the unsupplemented diet. In the study by Annison (1992) enzyme preparations containing xylanase and β-glucanase activities increased ileal pentosan (i.e. arabinoxylans) digestibility from 26% to

41% when added to a wheat-based diet. Following ileal digesta examination, it was noted that both soluble and insoluble pentosan concentrations were significantly lowered in birds fed enzyme-supplemented diets. This may be indicative of some disruption of the primary cell walls, leading to more efficient nutrient digestion by endogenous enzymes. In the study by Steenfeldt et al. (1998b), the total tract digestibility of NSP was improved significantly as a result of enzyme supplementation and a high positive correlation between AME_n and digestibility of NSP ($r = 0.88$) was noted. This suggests that the increased NSP digestibility and the subsequent microbial fermentation of NSP hydrolysis products in the hindgut may contribute energy to the chickens (Jorgensen et al., 1996).

The type and nutritive characteristics of wheat may affect responses to enzyme supplementation. Scott et al. (1998) demonstrated that wheat cultivars differed in their feeding values and poorer quality cultivars gave greater response to enzyme supplementation and vice versa. Such knowledge of variation in enzyme response could be used by feed manufactures to determine the economic merit of enzyme supplementation. Dusel et al. (1998) demonstrated that the effectiveness of xylanase addition depends on certain characteristics of the wheat variety (e.g. NSP content; in vitro and in vivo viscosity), with significant improvement in nutrient digestibility observed only for the pentosan-rich variety. However, Veldman and Vahl (1994) demonstrated that the positive effects of a xylanase was independent of wheat characteristics and the enzyme addition improved performance of broilers fed both high and low extract viscosity wheats, with no effect on intestinal viscosity.

The response to enzyme supplementation is also influenced by the age of the birds, with the greatest response shown by young birds. Leeson et al. (2000) demonstrated that

addition of commercial enzymes to wheat-based diets resulted in improved growth performance of broilers up to 4 wks. Such age dependent response was also noted in barley-based diets (Salih et al., 1991; Almirall and Esteve-Garcia, 1994). The digesta viscosity was high in barley-fed birds regardless of age, whereas the rate of feed passage was lower during the first 4 wks of age. This implies that the negative effect of viscosity on young birds is related to feed transit time. Older birds with a more mature digestive system appear capable of transporting the viscous material in the gastrointestinal tract more readily and therefore the viscosity reduction, owing to enzymes, results in less pronounced effects.

As indicated earlier, a multienzyme system is necessary to degrade NSP to a significant extent due to the complexity of the cell wall structure (Chesson, 1993; Slominski, 2000). Research conducted in our laboratory on Canadian wheat has shown that the addition of xylanase, β-glucanase and cellulase preparations to wheat-based diets significantly improves nutrient utilization and growth performance of broilers, whereas xylanase alone may not be as effective (Zhou, 2000). Therefore, there is a need for the use of multiple carbohydrase preparations to target various structures of wheat NSP for optimal nutrient utilization by broiler chickens. In addition, further research is necessary to identify situations when the enzyme utilization is most effective. This should improve the efficacy of carbohydrase enzymes and consistency of their effects when added to wheat-based diets.

2.5.2. Enzymes in Corn and SBM-based Diets

The use of feed enzymes to improve the nutritive value of corn and SBM-based diets has been examined in many studies. When targeting corn with a crude xylanase

preparation, Marquardt et al. (1994) failed to demonstrate a significant improvement in AME_n, nutrient digestibility, and growth performance of broiler chickens. However, Wyatt et al. (1999) found that enzyme addition increased the average ileal ME content of 8 different corn samples by 3.3% in broilers and reduced the variability in ileal ME among the corn samples by 50%.

Studies aimed at targeting the NSP of SBM with carbohydrase enzymes have shown variable results (Irish and Balnave, 1993; Marsman et al., 1997; Douglas and Parson, 2000; Kocher et al., 2002). Irish and Balnave (1993) found that the addition of two multiactivity enzyme preparations to corn and SBM-based diets resulted in a significantly poorer growth performance compared with an unsupplemented control diet. The authors concluded that soybean NSP were broken down and the presence of large amount of indigestible hydrolysis products, such as oligosaccharides, increased fluid retention in the small intestine and adversely affected nutrient absorption. On the other hand, Zanella et al. (1999) demonstrated that the addition of an enzyme mixture containing xylanase, protease and amylase to a corn-SBM broiler diet improved BW gain (1.9%) and feed-to-gain ratio (2.2%) as a result of increased ileal digestibility of protein, fat, and starch. Earlier research from our laboratory demonstrated that the use of multienzyme preparations in corn-SBM diets resulted in a consistent improvement in broiler chicken performance with the improvements in BW gain averaging 4.0% and feed-to-gain ratio 2.5% (Slominski, 2000). However, of 3 similar 2-wk growth performance experiments with broiler chickens, the statistically significant difference was only observed in 1 experiment for BW gain and in 2 experiments for feed-to-gain ratio. In another study, supplementation of a carbohydrase preparation to a semipurified diet

containing high concentration (38%) of SBM did not improve growth performance of broilers, although apparent ileal protein and NSP digestibility were increased significantly compared with the no enzyme treatment (Marsman et al., 1997). It was concluded that the enzymatic breakdown of both soluble and insoluble cell wall components may have contributed to the improvement in NSP digestibility. Douglas et al. (2000) evaluated 12 commercial SBM samples in a 2-wk broiler assay with or without enzyme supplementation. They also found that enzyme addition had no effect on chick growth performance but significantly increased ileal digestible energy content of the diets which contained 37% of the tested SBM. They also noted a significant interaction between SBM source and enzyme addition, indicating that the supplemental enzyme preparation increased dietary ileal DE more in some SBM samples than in the others. In a more recent study, Kocher et al. (2002) demonstrated that carbohydrase preparations containing glycanases (i.e. hemicellulases, cellulase) fortified with pectinase and galactanse activities increased NSP digestibility and AME content of diets containing high levels (36.5%) of SBM. Analysis of NSP content in ileal digesta revealed a partial depolymerization of both soluble and insoluble NSP of SBM. However, such beneficial effects did not result in an improvement in chick growth.

2.5.3. Enzymes in canola meal-based Diets

When using a carbohydrase cocktail of enzymes in a laying hen diet containing 40% CM, Slominski and Campbell (1990) demonstrated an increase in NSP digestibility. However, growth performance was not affected when similar enzyme products were added to broiler diets with high level of CM (Simbaya et al., 1996). More recently, Kocher et al. (2000) showed that a glycanase with hemicellulase and pectinase activities

reduced the soluble NSP concentration in the jejunum of broilers fed diets containing a high level (35%) of CM. However, such effect did not result in any significant improvement in nutrient utilization and growth performance.

2.5.4. Enzymes in Pea-based Diets

Brenes et al. (1993a) reported that addition of a cell-wall degrading multicarbohydrase complex improved feed-to-gain ratio of broiler chickens fed high-tannin but not low-tannin pea dies. Supplementation of broiler diets containing high levels of yellow, green, and brown peas with pectinase significantly improved BW gain and feed intake, whereas feed-to-gain ratio was not affected (Igbasan and Guenter, 1996). However, Igbasan et al. (1997b) observed that none of the growth performance parameters was affected when broiler chickens were fed a pea-based diet supplemented with graded levels of pectinase. Jeroch and Keller (1997) reported that a combination of α-galactosidase with a multicarbohydrase enzyme preparation improved the AME_n values of two varieties of peas. However, the addition of either enzyme alone resulted in very limited effects. The effects of supplementation with pectinase were also evaluated by Daveby et al. (1998) in broiler chickens fed diets containing 70% of dehulled peas. The enzyme addition did not show a significant effect on chick performance or apparent ileal digestibility of nutrients.

In conclusion, the cell wall degrading activities of carbohydrase enzymes seem to produce some beneficial effects in nutrient utilization and growth performance of broilers fed diets based on SBM, CM or peas. This often results from an increased NSP digestibility or altered concentrations of NSP fractions in the digesta of birds. Such observations indicate that the degradation of the cell wall NSP of these protein

supplements is possible despite the complex nature of the NSP polymers. The effectiveness of carbohydrase enzyme supplementation on nutrient utilization from full-fat oilseeds, such as flax and canola seed, would yet have to be determined.

2.6. Carbohydrase Enzyme Products, Substrate Hydrolysis, and Potentials for Further Improvement in Enzyme Efficacy

Dietary carbohydrase enzymes are primarily derived from bacterial and fungal fermentation with the latter acting as the source for many commercial enzyme supplements (Chesson, 1987). To date, most preparations in use were developed from industrial processes such as food and beverage processing. As such, the enzymes exist as relatively crude preparations, often containing many side activities in addition to their main activity. However, when applied to animal feeds, the presence of these side activities may contribute to the overall effectiveness of the enzyme preparation (Slominski, 2000).

Most carbohydrase enzymes of bacterial origin exhibit pH optima close to neutral. Fungal enzymes, on the other hand, generally exhibit maximal activity under more acidic conditions (pH 4.0-6.0). Most microbial enzymes should function most efficiently within the small intestine, where pH values are close to neutral, although lower pH conditions may be found in the upper areas of the small intestine close to the stomach.

Microorganisms (including fungi and bacteria) produce mainly 3 types of cellulase components, namely endocellulase, exocellulase and β-glucosidase, either as separate entities or in the form of an aggregated complex, for cellulose hydrolysis (Bhat and Bhat, 1997). In the hydrolysis of arabinoxylans, also 3 types of enzymes, i.e.,

endoxylanase (xylanase), β-xylosidase and α-L-arabinofuranosidase (Coughlan and Hazlewood, 1993), have been identified.

The mode of action of endocellulase, exocellulase (cellobiohydrolase), and β-glucosidase from several sources have been extensively studied (Bhat et al., 1990; Claeyssens and Henrissat, 1992). Based on these studies, for a complete hydrolysis of cellulose (Table 4), most endocellulases attack internal glycosidic bonds of cellulose and release mainly cellobiose and cellotriose. Exocellulase hydrolyses the second glycosidic bond from either the reducing or non-reducing end of the cello-oligosaccharides while β-glucosidase sequentially removes the glucose units from both ends.

Arabinoxylan hydrolysis (Table 4) is primarily accomplished by endoxylanase which cleaves the internal β-1,4 linkages of the xylan backbone and release mainly xylobiose, xylotriose and substituted oligomers having 2 to 4 residues (Coughlan and Hazlewood, 1993; Biely et al., 1997). Endoxylanase requires the presence of several unsubstituted xylosyl residues and therefore highly substituted fractions are resistant to enzyme hydrolysis. It has been reported that α-L-arabinofuranosidase activity significantly enhances the hydrolytic activity of xylanases by removing arabinose side chains (Coughlan and Hazlewood, 1993). β-xylosidase, on the other hand, enhances the appearance of xlosyl residues during enzymatic hydrolysis of arabinoxylans, presumably by the hydrolysis of xylo-oligomers.

Nearly all research done on the response of poultry to enzyme supplementation has used crude enzyme preparations with high levels of the desired activities, such as β-glucanase or xylanase, and with considerable amounts of other side activities. As such, many different carbohydrase preparations may have a number of the same activities

including xylanase, β-glucanase, cellulase etc. Such enzyme products, using xylanase as an example, may have different substrate preference and mode of action and may act in concert thereby enhancing the effectiveness of NSP degradation. Research carried out using different combinations of enzyme preparations may determine whether these enzyme preparations could complement each other and thus facilitate the efficacy of dietary enzyme supplementation. In this regard, it is known that endoxylanases cleave the xylose backbone of arabinoxylans. It has been reported that some bacteria produce endoxylanases that are specific for unsubstituted xylosidic linkages, while others produce endoxylanases that are specific for xylosidic linkages adjacent to substituted arabinose in the chain xylan (Coughlan et al., 1993). In addition, increased NSP degradation was demonstrated when 2 fungal cellulases were used in combination than when used alone (Wood et al., 1994). In the in vitro studies conducted in our laboratory (Slominski, 2000) a further improvement in the degree of wheat NSP hydrolysis was demonstrated when combinations of cell wall-degrading enzyme preparations (xylanase, β-glucanase, and cellulase preparations) were used. Similar in vitro incubation studies were carried out to determine if various enzyme preparations contained appropriate activities to target the cell wall polysaccharides in CM. A more pronounced depolymerization of the cell wall polysaccharides, as demonstrated by the increase in water-soluble NSP content, was achieved when three different enzyme preparations were used in concert.

TABLE 4. Enzyme systems involved in cellulose and arabinoxylan hydrolysis (Bhat and Hazlewood, 2001)

Enzyme	Substrate hydrolysis
Endocellulase or endoglucanase (1,4-β-D-glucan glucanohydrolase)	–G–G–G–G–G–G–G–G–G–G–G– ↑ ↑ Cleaves 1,4-β linkages at random
Exocellulase or cellobiohydrolase (1,4-β-D-glucan cellobiohydrolase)	G–G–G–G–G–G–G–G–G–G–G– ↑ (type I) ↑ (type II) ◀ Release cellobiose from both reducing (type II) and non-reducing ends (type I)
β-Glucosidase or cellobiase (β- D-glucoside glucohydrolase)	G–G; G–G–G–G; G–G–G–G–G ↑ ↑ ↑ ↑ ↑ Release glucose from cellobiose and hydrolyses short-chain cello-oligosaccharides from both reducing and non-reducing ends by releasing one glucose unit at a time
Xylanase or endoxylanase (1,4-β-D-xylan xylanohydrolase)	A A | | X–X–X–X–X–X–X–X–X–X–X–X–X– ↑ | ↑ A Cleaves 1,4-β linkages of xylan at random with preference to unsubstituted regions

3. MANUSCRIPT 1

Degradation of Cell Wall Polysaccharides by Combinations of Carbohydrase Enzymes and Their Effect on Nutrient Utilization and Broiler Chicken Performance

3.1. Abstract

In vitro incubation studies were carried out to determine if various carbohydrase preparations contained appropriate activities to target nonstarch polysaccharides (NSP) of wheat, soybean meal (SBM), canola meal (CM), and peas. Triplicate samples (0.1 g) were incubated with a number of carbohydrase preparations (i.e., cellulase, pectinase, xylanase, glucanase, galactanase and mannanase) or their combinations at 45°C and pH 5.2. A more pronounced degradation of NSP was achieved when the enzyme preparations were used in combination. When compared with the control (nonenzyme treatment), the highest degree of NSP degradation reached was 37% for wheat, and 36, 26, and 28% for CM, SBM and peas, respectively. Four enzyme combinations were studied further in a 2-wk (5 to 18 d of age) growth performance and nutrient digestibility trial with broiler chickens. All enzyme combinations were effective in improving ($P < 0.05$) weight gain, feed-to-gain ratio, AME_n, apparent ileal digestibilities of starch and protein, and apparent total tract digestibility of NSP in birds fed a wheat, wheat screening, SBM, CM, and peas-based diet. The most complex enzyme combination was found to be superior ($P < 0.05$) to others in improving ileal protein digestibility and feed-to-gain ratio. The effectiveness of this combination in elimination of the nutrient encapsulating effect of cell walls was further evaluated in a balance study with adult roosters fed a conventionally ground full-fat canola seed. Enzyme addition increased ($P < 0.05$) NSP digestibility from 11.1 to 30.1%, which, in turn, resulted in a marked increase ($P < 0.05$) in TME_n value (4.176 vs 4.744 Mcal/kg) of the seed. It is evident from the present studies that the addition of multicarbohydrase preparations to target cell wall polysaccharide

structures could further improve enzyme efficacy in practical wheat, SBM, CM, and peas-based broiler diets.

3.2. Introduction

Wheat is an important source of energy in poultry diets and contains from 8.3 to 9.8% of total nonstarch polysaccharides (NSP) (Slominski et al, 2000). Although arabinoxylans are the main polysaccharides of wheat (Henry, 1987), significant amounts of β-glucan and cellulose are also present (Steenfeldt et al., 1995). Nonstarch polysaccharide-degrading carbohydrases have been used in wheat-based diets for young poultry and their benefits have been credited to the partial breakdown of water-soluble and viscous arabinoxylans, which inhibit nutrient digestion and absorption by raising intestinal viscosity (Bedford and Classen, 1992). However, recent evidence suggests that the positive responses from enzyme supplementation are not always associated with a decrease in digesta viscosity (Veldman and Vahl, 1994; Dusel et al., 1998; Slominski et al., 2000; McCracken and Miller, 2002). The physical entrapment of wheat starch and protein by cell wall polysaccharides has been suggested as another important factor by which NSP exert their anti-nutritive properties (Theander et al., 1989; Bedford and Autio, 1996; Wiseman et al., 2000). In this context, the use of multicarbohydrase preparations to target various fractions of wheat NSP may provide a potential for further improvements in the nutritive value of wheat.

Due to high quality of protein, availability, and favorable price, canola meal (CM) and peas, along with soybean meal (SBM), are commonly used as sources of vegetable proteins in poultry diets. However, the optimum utilization of these products is often

influenced by their high content of NSP, which affects nutrient utilization (Bell and Keith, 1987; Longstaff and McNab, 1987; Dale, 1996). Total NSP content of 17.9% for CM (Slominski and Campell, 1990), 14.5% for SBM (Huisman et al., 1998), and 14.2% for peas (Igbasan et al., 1997b) has been reported. The major polysaccharides found in these feed ingredients are cellulose and pectic polysaccharides (Bach Knudsen, 1997). Attempts have been made to improve the utilization of these NSP for poultry by using different carbohydrase supplements. Although supplementation appears to be beneficial in improving NSP digestibility of CM (Slominski and Campbell, 1990; Simbaya et al., 1996; Kocher et al., 2000) and in enhancing NSP, protein and energy utilization from corn-SBM diets (Marsman et al., 1997; Doulglas et al., 2000; Kocher et al., 2002), such effects failed to result in better growth performance of poultry. Unlike SBM and CM, peas are richer in starch than protein (Castell et al., 1996). It has been shown that pea starch and protein are located within the cell wall matrix and the complex nature of cell wall structure makes these nutrients less accessible for endogenous enzymes of poultry (Longstaff and McNab, 1987). However, no conclusive effects of supplemental carbohydrase enzymes on growth rate or feed-to-gain ratio in pea-fed broilers were documented (Brenes et al., 1993a; Igbasan et al., 1997b; Daveby et al., 1998).

Full-fat canola seed has become an attractive feed ingredient in Canadian poultry diets because of its higher content of omega-3 unsaturated fatty acids, which can be deposited in egg or meat products and have a positive effect on human health. However, full utilization of the oil in canola seed by birds is limited because in the conventionally ground canola seed, a substantial amount of oil is encapsulated by cell wall polysaccharides. Sosulski and Sosulski (1993) demonstrated that cell wall hydrolyzing

enzymes facilitated the extraction of canola oil in the aqueous extraction process. However, such application of carbohydrase enzymes when feeding canola seed to poultry has not yet been investigated.

It is a common practice in the Canadian feed industry to partially substitute CM and peas for SBM in wheat-SBM based boiler diets for cost effectiveness. In such practical diets, which contain a number of plant ingredients and different forms of NSP, it is hypothesized that further improvements in nutrient utilization could be achieved using combinations of carbohydrases, each differing in their substrate preference and mode of action, to target various structures of cell wall polysaccharides. However, the information on utilization of such enzyme combinations in practical broiler diets is limiting (Rosen, 2000). Therefore, the objectives of this study were to screen several carbohydrase preparations for their ability to depolymerize the NSP of wheat, SBM, CM, and peas, and to evaluate the efficacy of enzyme combinations in a growth performance and nutrient utilization study with broilers fed a practical diet and in a balance study with roosters fed full-fat canola seed.

3.3. Material and Methods

3.3.1. In Vitro Enzyme Evaluation

An in vitro incubation study was carried out to determine if various carbohydrase preparations contained appropriate activities to target NSP of wheat, CM, SBM and peas, respectively. Enzyme preparations C (cellulase, 340 U/g), X (xylanase, 19,000 U/g), XG (xylanase, 63,600 U/g; glucanase, 48,300 U/g) and XG1 (xylanase, 47,500 U/g; glucanase, 50,350 U/g) were evaluated individually and in combination (C+X, C+XG,

and C+XG1) using wheat as a substrate. Enzyme preparations C, XG, P (pectinase, 10,000 U/g), G (galactanase, 1000 U/g), and MC (mannanase, 10,900 U/g; cellulase 600 U/g) were evaluated alone and in combination (C+P, C+XG, P+XG, C+P+XG, C+P+MC, C+P+XG+G, and C+P+XG+MC) using CM, SBM, and peas as substrates. Enzyme combinations were selected based on preliminary tests in which the degree of NSP degradation was determined. The enzyme activities reported above represent the main activities and do not reflect the side activities, which may have contributed to the degree of NSP depolymerizaiton. One unit of cellulase is the amount of enzyme which will produce 1 mg of reducing sugar (expressed as glucose) from carboxymethylcellulose per hour at 37°C and pH 4.6. One unit of xylanase activity was the amount of enzyme which will produce 1 μmole of reducing sugar (expressed as xylose) from arabinoxylan per minute at 40°C and pH 4.5. One unit of glucanase is the amount of enzyme which will liberate 1 mg of reducing sugar (expressed as maltose) from β-glucan per minute at 50°C and pH 5.0. One unit of mannanase is the amount of enzyme which will produce 1μmole of mannose reducing-sugar equivalents per minute at 40°C and pH 4.0. One unit of pectinase is the amount of enzyme which will liberate 1 μmole of galacturonic acid from polygalacturonic acid per minute at 25°C and pH 4.0. One unit of galactanase is the amount of enzyme which will produce 1μmole of galactose reducing-sugar equivalents per min at 40°C and pH 4.0. The enzyme preparations were provided, along with the enzyme assay procedures, by Canadian Bio-System Inc., Calgary, Alberta, Canada.

The in vitro incubation method applied in this study was the NSP analysis procedure described by Englyst and Cummings (1984, 1988). Triplicate samples (0.1 g) of wheat, CM, SBM, and peas were boiled at 100°C for 5 min with 6 mL of 0.1 *M*

sodium acetate buffer (pH 5.2) to gelatinize starch. Upon cooling, 1 mL of the buffer (control treatment) or 1 mL of enzyme solution was added followed by 1 mL of a solution of the starch-degrading enzymes (to remove starch). The amount of each enzyme preparation added was 0.01g/g of substrate. The mixtures were then incubated at 45°C for 16 h. Ethanol was added (to a final concentration of 80%) and the contents were mixed and allowed to stand for 1 h at room temperature. Following centrifugation at 3,000 rpm for 10 min, the supernatant containing ethanol-soluble enzyme hydrolysis products were discarded and the residue was subjected to NSP analysis as described under Chemical Analysis. The degree of cell wall polysaccharide degradation was indicated by a reduced recovery of total NSP and their constituent sugars compared with the control treatments. Effective enzyme combinations were selected for further evaluation in vivo.

3.3.2. Performance and Nutrient Utilization Study with Broilers

The efficacy of 4 enzyme combinations, C+P, C+XG, C+P+XG, and C+P+XG+MC, was evaluated in a growth performance and nutrient utilization experiment with broiler chickens. A wheat, wheat screening, SBM, CM, and peas-based diet (Table 5) was formulated to meet 95% of NRC requirement (1994) for AME and 92% for CP, calcium, available phosphorus, methionine, and methionine + cysteine. Other nutrients met or exceeded the NRC specifications. The diet was fed without enzyme supplementation or supplemented with each of the 4 combinations, giving five dietary treatments. Each enzyme preparation was added at a rate of 0.1 g/kg of diet. Chromic oxide (3.0 g/kg) was mixed with the diets and used to calculate the nutrient digestibilities and AME_n content.

TABLE 5. Composition and calculated analysis of basal diet

	g/kg
Ingredient	
Wheat (13.5% CP)	512.5
Soybean meal (46.5% CP)	185.0
Canola meal (36.0% CP)	70.0
Peas (22.0% CP)	50.0
Wheat screenings (15.0% CP)	100.0
Beef tallow	20.0
Crude canola oil	18.0
Limestone[1]	15.0
Dicalcium phosphate[2]	10.0
DL-methionine	0.9
L-lysine-HCl	0.6
Mineral premix[3]	5.0
Vitamin premix[4]	10.0
Chromic oxide	3.0
Total	1,000.0
Calculated analysis	
Crude protein (%)[5]	21.0
AME (kcal/kg)	3,040.0
Lysine (%)	1.12
Methionine (%)	0.47
Methionine + cysteine (%)	0.86
Calcium (%)	0.92
Available Phosphorous (%)	0.42

[1]Contained 38% calcium.

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

[3]Mineral premix provided per kilogram of diet: manganese, 55 mg; zinc, 50 mg; iron, 80 mg; copper, 5 mg; selenium, 0.1 mg; iodine, 0.36 mg; sodium, 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 feed composition data.

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[1] for a 4-d pre-experimental period and fed commercial chick starter crumbles (21% CP). On d 5, birds were fasted for 4 h, individually weighed and randomly distributed among the 5 treatments using 5 birds per pen and 9 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. BW 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 the 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 the analysis of gross energy, nitrogen, NSP, and chromic oxide contents. Nitrogen-corrected apparent metabolizable energy contents and total tract digestibility of NSP were calculated. On day 19, 24 birds 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 1cm above the ileo-caecal junction) were collected. Digesta samples from 4 birds within a treatment were pooled to yield 6 replicates per treatment. The ileal digesta samples were frozen, freeze-dried, ground and analyzed for nitrogen, starch, and chromic oxide to determine protein and starch digestibilities. Fresh digesta

[1] James Mfg. Co., Mount Joy, PA.

(1.5 g) from the jejunum were centrifuged at 9,000 rpm for 10 minutes and viscosity of the supernatant was determined at 40°C using the Brookfield digital viscometer[2].

3.3.3. Balance Study with Adult Roosters

The enzyme combination C+P+XG+MC was evaluated in a balance experiment with adult roosters fed full-fat canola seed. The sample of canola seed used in the study was obtained from a local egg producer in Manitoba, Canada. As a coarse grind of the seed was evident (particle size $\leq$2.0 mm), 3 samples of this material were used, including a subsample ground to pass through 1-mm sieve and 2 subsamples of the original coarse material without and with added enzyme (fine, coarse, and coarse + enzyme, respectively).

Nonstarch polysaccharide digestibility and TME_n were determined on the 3 samples using the assay described by Sibbald (1986) with some modifications (Zhang et al., 1994). Briefly, each sample was precision-fed (25 g per bird) to 3 groups of 10 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 and NSP contents. Pooled excreta from 30 birds fed 50 mL of a 50% glucose solution (25g of dry glucose) were used to determine the endogenous excretion of energy and nitrogen.

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

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[2] Model DV-II+LV, Brookfield Engineering Laboratories, Stoughton, MA.

3.3.4. 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[3] 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[4]. Starch was analyzed colorimetrically using Sigma Glucose (HK) 20 kit[5] and the procedure described by Aman and Hasselman (1984).

Nonstarch polysaccharide levels were determined by gas-liquid chromatography (component neutral sugars) and by colorimetry (uronic acids). The procedure for neutral sugars was performed as described by Englyst and Cummings (1984, 1988) with some modifications (Slominski and Campbell, 1990). Briefly, 100 mg feed or 50 mg digesta or excreta sample was boiled with 2 mL dimethylsulfoxide for 1 h and then incubated at 45°C overnight with a sodium acetate buffer solution (pH 5.2) containing the starch-degrading enzymes amylase, pullulanase, and amyloglucosidase[6]. Ethanol was then added 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 myoinositol (internal standard) solution were 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-

[3] LECO Corp., St. Joseph, MI.
[4] Parr Instrument Co., Moline, IL
[5] Sigma Chemical Co., St. Louis, MO.
[6] Sigma Chemical Co., St. Louis, MO.

methylimidazole. Component sugars were separated using a SP-2340 column and a Varian CP 3380 Gas Chromatograph[7]. Uronic acids were determined using the procedure described by Scott (1979).

3.3.5. Calculations and Statistical Analysis

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

Total tract NSP digestibility (%) = $\{1 - [(Cr_2O_3\ _{\%\ diet} / Cr_2O_3\ _{\%\ excreta}) \times (NSP\ _{\%\ excreta} / NSP\ _{\%\ diet})]\} \times 100$,

Ileal starch digestibility (%) = $\{1 - [(Cr_2O_3\ _{\%\ diet} / Cr_2O_3\ _{\%\ digesta}) \times (Starch\ _{\%\ digesta} / Starch\ _{\%\ diet})]\} \times 100$,

AME_n (kcal/kg of diet) = $GE\ _{kcal/kg\ diet} - [GE\ _{kcal/kg\ excreta} \times (Cr_2O_3\ _{\%\ diet} / Cr_2O_3\ _{\%\ excreta})] - 8.22 \times \{N\ _{\%\ diet} - [N\ _{\%\ excreta} \times (Cr_2O_3\ _{\%\ diet} / Cr_2O_3\ _{\%\ excreta})]\}$,

Where GE is gross energy, N is nitrogen, Cr_2O_3 is chromic oxide, and 8.22 is the energy equivalent of uric acid nitrogen, that is, 8.22 kcal/kg of uric acid nitrogen.

Both in vitro and in vivo studies were set up as completely randomized designs and data were subjected to ANOVA using the GLM procedure of SAS (SAS Institute, 1986) program. Means were separated by using Duncan's multiple range tests (Snedecor

[7] Varian Canada Inc., Mississauga, Ontario.

and Cochran, 1980). All statements of significance are based on a probability of less than 0.05.

3.4. Results

3.4.1. In Vitro Enzyme Evaluation

The effects of single and combined carbohydrases on degradation of the cell wall polysaccharides of wheat, CM, SBM, and peas are presented in Tables 6, 7, 8, and 9, respectively. The total NSP content of wheat averaged 87.8 g/kg with arabinose (23%), xylose (28%) and glucose (41%) accounting for the major constituent sugars. Mannose and galactose were also present, but in small quantities. Much higher amounts of total NSP were found in CM (171 g/kg), SBM (148 g/kg), and peas (127 g/kg) compared with wheat. Glucose, uronic acids, galactose, arabinose, and xylose were the predominant constituent sugars in these feedstuffs, although the proportion of these sugars differed among the 3 feedstuffs. Glucose accounted for 31% of the total NSP content and was the major constituent sugar in CM, followed by uronic acids, arabinose, xylose, and galactose. In contrast to CM, 30% of SBM NSP was galactose with glucose, uronic acids, arabinose and xylose detected at appreciable amounts. When compared with SBM and CM, pea NSP contained a much higher amount of glucose (57%), and relatively lower amounts of uronic acids, arabinose, xylose and galactose. Small amounts of mannose were noted for all the 3 feedstuffs.

Incubation of wheat with enzyme preparations C, X, XG, and XG1 resulted in a significant ($P < 0.05$) degree of NSP degradation (Table 6) that ranged from 22 to 28%. Similar breakdown patterns of NSP were observed for each of the enzyme preparations

TABLE 6. Degradation of nonstarch polysaccharides (NSP) following incubation of wheat with different carbohydrase preparations (g/kg)[1]

Enzyme	Component sugar					Total NSP
	Arabinose	Xylose	Mannose	Galactose	Glucose	
None (control)	19.9[a]	24.7[a]	3.6	3.4	36.2[a]	87.8[a]
Cellulase (C)	12.2[cd]	15.9[b]	2.8	3.2	31.1[bc]	65.2[cd]
Xylanase (X)	14.7[b]	14.7[c]	2.9	3.7	32.8[b]	68.7[b]
Xylanase/glucanase (XG)	12.3[cd]	13.1[cd]	3.3	3.1	31.3[bc]	63.2[d]
Xylanase/glucanase1 (XG1)	14.8[b]	15.8[b]	2.8	3.2	31.3[bc]	67.9[bc]
C + X	13.1[bc]	12.3[cd]	3.3	3.8	29.4[cd]	62.0[d]
C + XG	10.6[d]	11.0[d]	2.8	2.9	27.8[d]	55.0[f]
C + XG1	10.9[d]	11.3[d]	2.9	3.4	29.8[cd]	58.3[e]
SEM	0.67	0.78	0.33	0.39	0.81	1.17

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

[1] Means of triplicate determination.

TABLE 7. Degradation of nonstarch polysaccharides (NSP) following incubation of canola meal with different carbohydrase preparations (g/kg)[1]

Enzyme	Component sugar						Total NSP[2]
	Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acids	
None (control)	31.5^{a}	15.4^{a}	4.2	14.2^{a}	53.5^{a}	47.0^{a}	171^{a}
Cellulase (C)	27.8^{b}	13.8^{bc}	3.8	14.1^{a}	41.4^{d}	46.9^{a}	153^{cd}
Pectinase (P)	24.3^{cd}	15.2^{ab}	4.0	11.8^{c}	53.4^{a}	41.0^{de}	155^{cd}
Galactanase (G)	31.5^{a}	14.7^{abc}	4.0	13.3^{ab}	53.5^{a}	45.0^{abc}	167^{ab}
Xylanase/glucanase (XG)	25.2^{bc}	13.3^{cd}	4.2	12.3^{bc}	52.6^{ab}	46.3^{ab}	159^{bc}
Mannanase/cellulase (MC)	18.7^{ef}	15.3^{ab}	4.2	14.1^{a}	49.0^{bc}	46.9^{a}	154^{cd}
C+P	21.3^{de}	11.2^{ef}	3.6	11.0^{cd}	37.1^{e}	40.7^{de}	130^{fg}
C+XG	21.6^{de}	11.3^{ef}	4.1	11.7^{c}	33.7^{ef}	46.4^{ab}	134^{ef}
XG+P	20.1^{e}	12.2^{de}	4.1	11.1^{cd}	48.7^{c}	43.2^{bcd}	144de
C+P+XG	17.2^{f}	9.9^{fg}	3.9	10.0^{de}	34.4^{ef}	42.1^{cde}	122^{g}
C+P+MC	17.9^{ef}	9.4^{g}	3.9	9.6^{e}	35.8^{e}	41.0^{de}	121^{g}
C+P+XG+G	18.7^{ef}	10.6^{fg}	4.1	11.1^{cd}	34.0^{ef}	42.7^{cd}	126^{fg}
C+P+XG+MC	12.6^{g}	9.1^{g}	4.0	8.9^{e}	31.1^{f}	38.7^{e}	109^{h}
SEM	1.14	0.53	0.22	0.50	1.27	1.20	4.0

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

[1]Means of triplicate determination.

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

TABLE 8. Degradation of nonstarch polysaccharides (NSP) following incubation of soybean meal with different carbohydrase preparations (g/kg)[1]

Enzyme	Component sugars						Total NSP[2]
	Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acids	
None (control)	20.2[a]	12.0[a]	5.6	44.0[a]	34.9[a]	27.9[a]	148[a]
Cellulase (C)	17.3[bc]	10.5[b]	5.2	44.1[a]	31.5[b]	27.4[ab]	139[ab]
Pectinase (P)	16.9[bc]	11.1[ab]	5.1	38.8[b]	34.9[a]	24.5[cde]	135[bc]
Galactanase (G)	20.0[a]	10.8[a]	5.1	42.8[a]	34.8[a]	25.7[abc]	144[ab]
Xylanase/Glucanase (XG)	17.2[b]	10.4[b]	5.0	37.2[bc]	34.9[a]	27.4[ab]	136[bc]
Mannanase/Cellulase (MC)	15.6[bcd]	11.6[a]	5.6	43.9[a]	31.2[b]	27.5[ab]	139[ab]
C+P	15.6[bcd]	10.2[bc]	5.2	37.8[b]	30.2[b]	24.8[bcd]	127[cd]
C+XG	15.5[bcd]	10.4[b]	5.5	36.5[bc]	29.4[b]	27.2[abc]	128[cd]
XG+P	16.4[bc]	9.2[d]	5.5	36.5[bc]	32.4[ab]	24.8[bcd]	128[cd]
C+P+XG	14.5[cde]	9.2[d]	5.3	36.3[bc]	30.9[b]	22.6[de]	122[d]
C+P+MC	13.9[cde]	9.0[d]	5.5	35.4[bc]	30.4[b]	23.7[cde]	120[d]
C+P+XG+G	14.6[cde]	9.0[d]	5.4	35.1[bc]	30.2[b]	25.0[bcd]	123[d]
C+P+XG+MC	12.1[e]	8.9[d]	5.3	32.7[c]	26.1[c]	22.0[e]	110[e]
SEM	0.86	0.36	0.26	1.34	1.10	0.93	3.3

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

[1]Means of triplicate determination.

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

TABLE 9. Degradation of nonstarch polysaccharides (NSP) following incubation of peas with different carbohydrase preparations (g/kg)[1]

Enzyme	Component sugars						
	Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acids	Total NSP[2]
None (control)	16.8[a]	8.6[a]	2.5	5.0[a]	70.3[a]	20.5[a]	127[a]
Cellulase (C)	13.5[b]	7.8[ab]	2.4	5.0[a]	63.1[cde]	20.0[ab]	115[bc]
Pectinase (P)	10.3[c]	8.5[a]	2.4	3.1[de]	68.9[ab]	17.6[cd]	114[bcd]
Galactanase (G)	16.6[a]	8.5[a]	2.5	4.8[ab]	69.4[ab]	19.8[ab]	125[a]
Xylanase/Glucanase (XG)	13.4[b]	7.6[b]	2.5	4.3[bc]	68.7[ab]	19.8[ab]	119[ab]
Mannanase/Cellulase (MC)	8.7[de]	8.3[ab]	2.3	5.0[a]	65.5[bcd]	20.3[ab]	113[bcd]
C+P	9.8[cd]	7.5[b]	2.5	3.2[d]	61.3[def]	18.4[bc]	106[def]
C+XG	9.0[cde]	6.0[c]	2.5	4.2[c]	59.4[ef]	20.3[ab]	104[efg]
XG+P	8.2[ef]	6.0[c]	2.4	3.2[d]	66.4[bc]	17.5[cd]	107[cde]
C+P+XG	7.7[ef]	5.4[c]	2.3	2.6[ef]	57.8[f]	16.9[d]	96[gh]
C+P+MC	7.7[ef]	5.9[c]	2.4	2.6[ef]	57.5[f]	16.7[d]	93[h]
C+P+XG+G	7.2[fg]	5.3[c]	2.2	2.5[f]	60.0[ef]	17.7[cd]	98[fgh]
C+P+XG+MC	6.0[g]	4.0[d]	2.1	2.3[f]	57.0[f]	17.0[cd]	91[h]
SEM	0.49	0.30	0.15	0.18	1.49	0.70	3.0

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

[1]Means of triplicate determination.

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

because the reduced recoveries of total NSP resulted from the removal ($P < 0.05$) of xylose, arabinose, and to a lesser extent, glucose. Combining the enzyme preparation C (cellulase) with X (xylanase), XG (xylanase/glucanase), or XG1 (xylanase/glucanase) led to a more pronounced degradation of wheat NSP ($P < 0.05$), with the combination C+XG contributing to the highest ($P < 0.05$) NSP degradation.

When compared with a control treatment, incubation with enzyme preparation C reduced ($P < 0.05$) the recovery of glucose, arabinose, and xylose in CM and SBM, and of glucose and arabinose in peas. However, total NSP degradation was significant ($P < 0.05$) only for CM (10.5%) and peas (9.4%) (Tables 7, 8, 9). Enzyme P (pectinase) brought about an NSP degradation ($P < 0.05$) ranging from 8.8 to 10.2% for the 3 substrates and the degradation originated mainly from the reduced recoveries ($P < 0.05$) of uronic acids, arabinose and galactose. Incubation with enzyme XG reduced ($P < 0.05$) the recoveries of arabinose, xylose, and galactose for each feedstuff, leading to NSP degradation ($P < 0.05$) in CM (7.0%) and SBM (8.1%), but not in peas. The recoveries of arabinose and glucose decreased ($P < 0.05$) with enzyme MC (mannanase/cellulase) for each substrate, although the total NSP degradation was only significant ($P < 0.05$) for CM (9.9%) and peas (11.0%). In contrast, enzyme G (galactanase) did not affect ($P < 0.05$) the recoveries of NSP from any of the targeted substrates.

Unlike the effect of enzymes applied singly, incubation of CM, SBM, and peas with the enzyme combinations generally decreased ($P < 0.05$) the recoveries of all the constituent sugars, except mannose. As was the case for wheat, the reduced recoveries of total NSP were more pronounced when the enzyme preparations were used in concert. The lowest NSP recoveries were observed for the combination C+P+XG+MC, with a

degree of NSP degradation averaging 36, 26, and 28% for CM, SBM and peas, respectively. The efficacy of combination C+P+XG+MC was the highest ($P < 0.05$) among the combinations evaluated using CM and SBM, but was similar to the combinations C+P+XG and C+P+XG+G when the substrate was peas.

3.4.2. Broiler Performance and Nutrient Utilization Study

The growth performance of broilers during the 2-wk experiment and AME_n contents of experimental diets are shown in Table 10. Feed consumption was not affected ($P > 0.05$) by dietary enzyme supplementation. However, BW gain of broilers fed diets containing different combinations of carbohydrases was greater ($P < 0.05$) than that of birds fed the control diet. However, no differences ($P > 0.05$) in BW gain were observed among enzyme-supplemented diets. Feed-to-gain ratio was markedly improved ($P < 0.05$) by enzyme addition. Among the enzymes evaluated, supplementation with enzyme C+P+XG+MC produced the highest improvement ($P < 0.05$) in feed-to-gain ratio. Nitrogen-corrected apparent metabolizable energy contents of enzyme-supplemented diets were higher ($P < 0.05$) compared with the control diet and no difference ($P > 0.05$) among enzyme combinations was noted.

The utilization of NSP, starch, and protein, and intestinal digesta viscosity are summarized in Table 11. The apparent total tract digestibility of NSP was similar ($P > 0.05$) among the enzyme-supplemented diets (12.8-14.9%) and was higher ($P < 0.05$) than that of the control diet (6.3%). Likewise, jejunal digesta viscosity of the chicks was reduced ($P < 0.05$) from 3.3 (control) to 2.2 or 2.3 mPa·s by inclusion of different combinations of carbohydrase enzymes. When compared with the control diet, ileal digestibilities of starch and protein in birds fed enzyme-supplemented diets were

TABEL 10. Growth performance of broiler chickens (5 to 18 d) and AME_n content of diets supplemented with different combinations of carbohydrase preparations

Enzyme[1]	Feed intake[2] (g/bird)	Body weight gain[2] (g/bird)	Feed to gain ratio[2]	AME_n[3] (kcal/kg)
None (control)	668	436[b]	1.53[a]	2,902[b]
C+P	687	459[a]	1.50[b]	2,997[a]
C+XG	695	470[a]	1.48[b]	3,004[a]
C+P+XG	678	456[a]	1.49[b]	3,001[a]
C+P+XG+MC	676	466[a]	1.45[c]	3,046[a]
SEM	9.5	6.5	0.009	21.0

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

[1]Main enzyme activities in enzymes: C = cellulase; P = pectinase; XG = xylanase and glucanase; MC = mannanase and cellulase. All enzymes were added at 0.1 g/kg of respective diet.

[2]Means of 9 replicate pens of 5 birds each.

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

TABLE 11. Apparent total tract digestibility of nonstarch polysaccharides (NSP), jejunal digesta viscosity, and apparent ileal digestibility of starch and protein of broiler chickens fed diets supplemented with different combinations of carbohydrase preparations

me[1]	NSP[2] (%)	Viscosity[3] (mPa s)	Starch[3] (%)	Protein[3] (%)
None (control)	6.3[b]	3.3[a]	92.6[c]	73.2[c]
C+P	14.0[a]	2.3[b]	94.7[b]	76.3[b]
C+XG	14.0[a]	2.2[b]	95.7[ab]	77.5[b]
C+P+XG	12.8[a]	2.3[b]	95.6[ab]	77.2[b]
C+P+XG+MC	14.9[a]	2.2[b]	96.7[a]	79.8[a]
SEM	1.37	0.12	0.49	0.72

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

[1]Main enzyme activities in enzymes: C = cellulase; P = pectinaese; XG = xylanase and glucanase; MC = mannanase and cellulase. All enzymes were added at 0.1 g/kg of respective diet.

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

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

significantly ($P < 0.05$). Among the enzyme treatments, supplementation with combination C+P+XG+MC further improved ($P < 0.05$) starch digestibility from that of enzyme C+P and protein digestibility ($P < 0.05$) from those of other enzyme combinations.

3.4.3. Adult Rooster Balance Study

The results of the effect of particle size and enzyme supplementation on TME_n and NSP digestibility in adult roosters fed full-fat canola seed are depicted in Figures 2 and 3. The TME_n of the conventionally ground (coarse) canola seed was markedly improved ($P < 0.05$) by reducing the particle size (to fine) or by supplementing with thecarbohydrase combination C+P+XG+MC. Although the birds given the finely ground canola seed had a similar NSP digestibility ($P > 0.05$) to those fed the original coarsely ground seed (11.1 vs. 9.9%), supplementation with the carbohydrase combination increased ($P < 0.05$) the NSP digestibility (from 11.1 to 30.1%) in coarsely ground canola seed.

3.5. Discussion

The amount of total NSP in the wheat used in the present study was in the range (8.3 to 9.8%) reported previously for Canadian wheat by Slominski et al. (2000) and was similar to that found in wheat from other countries (Austin et al., 1999; Steenfeldt, 2001; Pirgozliev et al., 2003). The constituent sugar profile confirmed that arabinoxylans (estimated from the sum of arabinose and xylose) account for approximately 50% of the total NSP content of wheat and are the major polysaccharides of the wheat endosperm cell walls (Henry, 1987). Aman (1988) suggested that wheat arabinoxylan consists of a

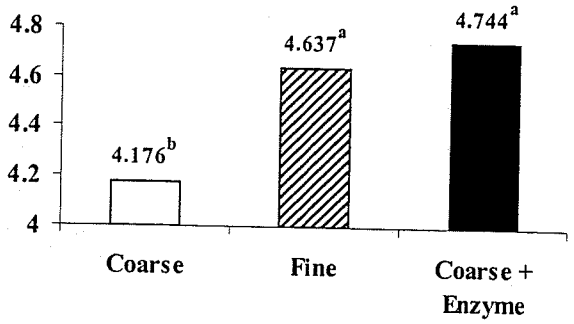

FIGURE 2. Effect of particle size and enzyme supplementation on TME_n content (Mcal/kg dry matter) of full-fat canola seed fed to adult roosters. Coarse = coarsely ground canola seed (particle size ≤2.0 mm) obtained from a local egg producer; Fine = coarse canola seed ground to pass through a 1-mm sieve; Coarse + enzyme = coarse canola seed supplemented with 4 carbohydrase preparations containing cellulase, pectinase, xylanase, glucanase, and mannase as main activities. [a,b]Means with no common superscript differ ($P < 0.05$), and each mean was from 3 pooled sample of 10 birds each. The pooled SEM was 0.105.

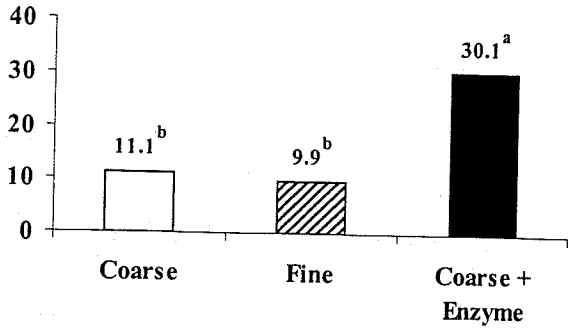

FIGURE 3. Effect of particle size and enzyme supplementation on nonstarch polysaccharides (NSP) digestibility (%) in adult roosters fed full-fat canola seed. Coarse = coarsely ground canola seed (particle size ≤2.0 mm) obtained from a local egg producer; Fine = coarse canola seed ground to pass through a 1- mm sieve; Coarse + enzyme = coarse canola seed supplemented with 4 carbohydrase preparations containing cellulase, pectinase, xylanase, glucanase, and mannase as main activities. [a,b]Means with no common superscript differ ($P < 0.05$), and each mean was from 3 pooled sample of 10 birds each. The pooled SEM was 2.56.

xylose backbone substituted with 1 or 2 terminal arabinose residues. The amount of glucose was also high, indicating the presence of both β-glucan and cellulose (Henry, 1987) primarily in the aleurone layers and pericarp or testa of the grain (Steenfeldt et al., 1995).

The amounts of total NSP reported for CM, SBM, and peas in the literature varies markedly, presumably due to the differences in analytical techniques, growing conditions, variety or, as may be the case for soybean, the degree of hull removed. However, the component sugar profiles observed in the current study agree with thosereported earlier (Slominski and Campbell, 1990; Daveby and Aman, 1993; Huisman et al., 1998). The sugar profiles confirmed that the main polysaccharides of CM, SBM, and peas are pectic polysaccharides (Bach Knudsen, 1997) with uronic acids, arabinose and galactose residues predominating. The characteristic structure of pectic polysaccharides comprises a main chain of rhamnogalacturonan consisting of galacturonic acid and rhamnose residues, side chains containing arabinose, galactose, and xylose residues, as well as highly branched arabinans, galactans, and arabinogalactans present as side chains or free neutral polysaccharides (Aspinall and Cottrell, 1971; Siddiqui and Wood, 1972; Daveby and Aman, 1993). Other major polysaccharides consisting of glucose, xylose and arabinose residues could include cellulose, glucoxylans and glucoarabinoxylans. β-Mannans appeared to be the minor components as indicated by the low mannose content.

It has been suggested that the action of carbohydrase enzymes is mediated by degrading high molecular weight polysaccharides to simple sugars, oligosaccharides, and low molecular weight polysaccharides (Slominski et al., 1993; Castanon et al., 1997),

which contributed to the reduced recovery of NSP when the feedstuff samples were incubated with enzymes in the current in vitro study. Using wheat as the substrate, the reduced recoveries of arabinose, xylose and glucose following incubation with the individual enzymes indicate that each of the enzyme preparations contained the arabinoxylan-, β-glucan-, and cellulose-degrading activities, despite their main claimed activities. This is not surprising given that most enzyme sources used in animal feeds are crude products (Bedford and Classen, 1993) and most microorganisms used for enzyme production are capable of producing multiple activities (Bhat and Hazlewood, 2001). This highlights the advantage of using feed ingredients as substrates to assess the effectiveness of enzyme preparations over the conventional methods in which purified substrates from different sources are used. The latter scenario could lead to erroneous conclusions regarding the expected effectiveness of the enzyme preparation.

When using CM, SBM, and peas as substrates, a similar constituent sugar recovery pattern arose from each of the carbohydrase preparations evaluated, which may be explained by some similarity in the form and structure of the polysaccharides present in these feedstuffs (Bach Knudsen, 1997). In addition, the relatively lower degree of NSP degradation (~10%) following incubation of CM, SBM, and peas with single enzymes reflects the structural complexity of the NSP fraction being less degradable than that of wheat (22 - 28%). Because of the heterogenous nature of the NSP fraction, it is difficult to determine the specific mode of action of each enzyme from the recovered component sugar profiles only. However, it appears that both enzyme preparations C (cellulase) and MC (mannanase and cellulase) acted on the insoluble cellulose, glucoxylans and glucoarabinoxylans, as shown by the reduced recoveries of glucose, arabinose and

xylose. Contrary to its action on wheat NSP, preparation XG (xylanase and glucanase) may have depolymerized free arabinogalactan (arabinose and galactose) or rhamnogalacturonan side chains (arabinose, galactose and xylose) rather than the backbone, as illustrated by the unaltered recoveries of uronic acids. From the reduced recoveries of uronic acids, arabinose, and galactose following incubation with enzyme P (pectinase), it can be speculated that this enzyme contained multiple activities towards pectic polysaccharides, acting not only on the backbone but also on the side chains and the free neutral polymers. Enzyme G (galactanase) was not effective in degrading the NSP of any substrates used and enzyme MC did not depolymerize β-mannans (the recovery of mannose from each feedstuff was unaffected). These results further document that the use of pure and specific substrates in enzyme evaluation may lead to false conclusions.

The results of the in vitro study using four feedstuffs commonly used in Canadian broiler diets demonstrated that various carbohydrase preparations were generally more effective in the degradation of cell wall polysaccharides when used in combination, and that the extent of NSP degradation depended on the combinations used. As discussed above, each individual enzyme evaluated for wheat revealed a full spectrum of xylanase, β-glucanase and cellulase activities. However, the improved effectiveness of enzyme combinations could not be attributed to the increases in enzyme activities since high dosages (1%) of enzymes were used and increases in enzyme activity level produced no further NSP degradation in the preliminary assay (data not shown). Rather, each individual preparation produced by different microorganisms may have had different substrate preference and mode of action and could have acted in concert thereby

enhancing the effectiveness of NSP degradation. In this regard, it is known that endoxylanases cleave the xylose backbone of arabinoxylans. It has been reported that some bacteria produce endoxylanases that are specific for unsubstituted xylosidic linkages, while others produce endoxylanases that are specific for xylosidic linkages adjacent to substituted arabinose in the xylan chain (Coughlan et al., 1993). In addition, NSP degradation was demonstrated to be more effective when 2 fungal cellulases were used in combination (Wood et al., 1994). The results from using CM, SBM, and peas as substrates lend further support for the above supposition. Only cellulose and xylan fractions or pectic polysaccharides were depolymerized when enzymes were used individually, whereas both types of polysaccharides were hydrolyzed when using enzyme combinations. The greatest effect observed with combinations C+P+XG+MC for CM, SBM, and peas, and C+XG for wheat, may be indicative of the variety of carbohydrase activities present, which complemented one another in cleaving various linkages in the complex polysaccharide structures.

The broiler study demonstrated that the 4 enzyme combinations were effective in improving nutrient utilization, AME_n content and growth performance of birds fed a wheat-SBM based diet that also contained significant amounts of CM and peas. Because the basal diet also included 10% wheat screenings, which generally contain 67-84% of crushed and small kernel wheat (Stapleton et al., 1980), the total wheat content of the diets was close to 60% as is the case for any practical Canadian broiler diets. To our knowledge, no comparable published data on the influence of carbohydrase enzymes on such complex practical diets is available. The results of the current study further support earlier reports that the addition of xylanase, β-glucanase and cellulase preparations to

wheat-based diets significantly improves nutrient utilization and growth performance of broilers, whereas xylanase alone may not be as effective (Chesson, 1993; Zhou, 2000). Therefore, the results of the current study support the need for the use of multiple carbohydrase preparations to target various structures of wheat NSP for optimal nutrient utilization by broiler chickens. In addition, this study demonstrated that the most effective enzyme combination in vitro (i.e., C+P+XG+MC) was superior to the other enzyme combinations only in improving protein digestibility and feed-to-gain ratio. How these additional improvements were achieved can not be interpreted from the NSP digestibility, which was similar to that of other enzyme combinations. This is contrary to the results of the in vitro study in which the enzyme combination C+P+XG+MC showed the highest degree of NSP degradation for CM, SBM and peas.

It is known that wheat soluble NSP increase intestinal viscosity. A higher intestinal viscosity slows the diffusion rate of substrates, digestive enzymes, and digestion end products and consequently affects nutrient digestion and utilization (Petterson and Aman, 1989). Carbohydrase enzymes are capable of partially degrading soluble NSP into smaller molecular weight polymers and thus decrease digesta viscosity. This, in turn, improves nutrient utilization and animal performance (Simon, 1998). Hence, the reduction in viscosity has been suggested in many studies as the main reason for improved growth performance and nutrient utilization in the enzyme-supplemented wheat-based diets (Bedford and Classen, 1992; Marquardt et al., 1994). The intestinal viscosity observed in the current study derived primarily from the water-soluble wheat arabinoxylans and β-glucans because the polysaccharides of CM, SBM, and peas are minor contributors to digesta viscosity (Bach Knudsen, 1997). It has to be emphasized,

however, that in the current experiment a relatively low digesta viscosity value was found for the control birds (3.3 mPa·s). Low viscosity values were reported by other authors in studies utilizing Canadian wheat-based diets containing 60% or more of wheat (Leeson et al., 2000; Slominski et al., 2000; McCracken and Miller, 2002). Therefore, the extent to which a further reduction in digesta viscosity contributed to the responses from dietary carbohydrase addition can not be determined from the current study. It seems rather unlikely that the intestinal viscosity alone was responsible for the observed differences in growth performance. This is particularly true for treatment with the enzyme combination C+P+XG+MC, which was superior to the other enzyme treatments although no change in jejunal viscosity was observed. Similar findings, in which the responses from enzyme supplementation were not associated with digesta viscosity, have been reported (Veldman and Vahl, 1994; Dusel et al., 1998, McCracken and Miller, 2002). It is noteworthy such responses from enzyme supplementation generally occurred for wheat of relatively low viscosity, suggesting that other factors may be of importance in situations when digesta viscosity is low.

The disruption of cell wall integrity and release of encapsulated nutrients most likely contributed to the overall improvements with carbohydrase enzyme supplementation observed in the current broiler study. In this regard, Classen (1996) suggested that wheat NSP may act as a physical barrier preventing or slowing access of endogenous enzymes to starch granules. The microscopy study by Bedford and Autio (1996) demonstrated that there was indeed a considerable amount of starch surrounded by intact cell walls in the intestinal digesta of broilers fed wheat-based diets, which was largely removed on addition of an NSP-degrading carbohydrase. The starch and protein

in peas are encapsulated by the cell wall polysaccharides and thus less available for digestion (Wursch et al., 1986; Longstaff and McNab, 1987; Daveby et al., 1998). Supplemental carbohydrases may partially depolymerize the NSP of SBM (Marsman et al., 1997; Kocher et al., 2002) or CM (Kocher et al., 2000), thereby improving protein digestibility (Marsman et al., 1997; Kocher et al., 2002). Another potential mode of action of carbohydrase enzymes could be the hydrolysis of certain types of carbohydrate protein complexes (i.e., glycoproteins, proteoglycans) in which the protein component is resistant to proteolysis because of its substitution with bulky carbohydrate groups. This could have contributed to some improvements in protein digestibility observed in the current study when the most potent carbohydrase combination C+P+XG+MC was used. Such an effect could be valid for canola and soybean, which undergo a significant seed disruption during the prepress solvent extraction process with the resulting meal lacking most of the original cell integrity. Thus, it is less likely CM and SBM would contain any significant amount of nutrients not available for digestion due to encapsulating effect of the cell walls.

The net improvements produced by the most effective enzyme combination C+P+XG+MC were 4%, 9%, 144 kcal/kg, 8%, and 8 units, respectively, for starch digestibility, protein digestibility, AME_n content, weight gain and feed-to-gain ratio. However, it is not clear which feed ingredient contributed to such improvements and to what extent. Further research is needed to investigate the effect of enzyme C+P+XG+MC on each individual ingredient using purified diets containing wheat, SBM, CM, or peas.

Overall, the elimination of the encapsulating effect and, to some extent, the reduction of digesta viscosity contributed to the beneficial effects of carbohydrase

supplementation observed in the current broiler study. Regardless of the mechanism involved, the net benefit of carbohydrase application would be an improved rate of nutrient digestion and utilization in the upper gut of birds, leaving less substrates available for microbial fermentation in the lower gut (Choct et al., 1996). This means not only more efficient nutrient utilization by the animal (Uni et al., 1999) but also healthier birds, because the microbial challenge for the animal would be reduced due to a limited substrate availability (Bedford, 2000).

It is known that the oil in canola seed is located within numerous cells of the cotyledons, which are surrounded by a thick wall of polysaccharides (Sosulski and Sosulski, 1993). However, due to the small size, grinding may not effectively disrupt the polysaccharide cell wall of canola seed. This is supported by the considerable increase in TME_n content observed in the balance study for both the finely ground seed and the enzyme-supplemented coarsely ground seed. This indicates that the combination of 4 carbohydrase enzymes (C+P+XG+MC) was as effective as physical grinding in the disruption of the cell wall structure, leading to the release of oil, the major contributor to the TME_n value. The disruption of the cell wall structure was further substantiated by the markedly increased NSP digestibility by the roosters upon the addition of the carbohydrase combination. Hence, such a combination of carbohydrase preparations may serve as a means to further improve energy utilization from full-fat canola seed when fed to poultry. Further research is needed to validate its effectiveness in complete poultry diets.

In conclusion, the present studies indicate that the selected carbohydrase preparations in combination were effective in degrading cell wall polysaccharides in vitro

and in improving growth performance and nutrient utilization in vivo. The beneficial effects of such combinations observed in the broiler study may have resulted from elimination of the nutrient encapsulating effect of the cell wall polysaccharides and, to some extent, from the reduction of intestinal viscosity. This suggests that application of an appropriate combination of carbohydrase enzymes to target various structures of cell wall polysaccharides could further improve the efficacy of the existing enzyme products in practical wheat, SBM, CM, and peas-based broiler diets.

3.6. Acknowledgements

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4. MANUSCRIPT 2

Nutritive Values of Corn, Soybean Meal, Canola Meal and Peas for Broiler Chickens as Affected by a Multicarbohydrase Preparation of Cell Wall Degrading Enzymes

4.1. Abstract

The effect of a multicarbohydrase supplement of cell wall degrading activities on the nutritive value of corn, soybean meal (SBM), canola meal (CM), and peas for broiler chickens was investigated. Four isoenergetic and isonitrogenous corn (69% corn), SBM (30% SBM, 59% corn), CM (30% CM, 54% corn) and pea (30% peas, 52% corn) diets, without or with enzyme supplementation, were formulated to meet NRC specifications for broiler chickens (except for AME and CP which were at 95 and 92% NRC requirements, respectively). The enzyme supplement supplied 1,000U xylanase, 400U glucanase, 1,000U pectinase, 120U cellulase, 280U mannanase, and 180U galactanase per kilogram of diet. Each diet was fed in a mash form to 9 replicate pens of 5 broilers from 5 to 18 d. When compared with the control treatment, enzyme addition to the corn diet improved ($P < 0.05$) feed-to-gain ratio whereas the performance of birds fed the other 3 diets was not affected. An increase ($P < 0.05$) in total tract nonstarch polysaccharides (NSP) digestibility, ileal starch digestibility, and AME_n was observed in birds fed the enzyme-supplemented corn diet. An improvement ($P < 0.05$) in total tract NSP digestibility, ileal protein digestibility, and AME_n content with enzyme supplementation was observed for the SBM diet. However, nutrient digestibilities and AME_n of CM and pea diets were not affected ($P > 0.05$) by enzyme addition even though the NSP digestibilities increased significantly ($P < 0.05$). A significant increase ($P < 0.05$) in water-soluble NSP and a decrease ($P < 0.05$) in water-insoluble NSP concentration of ileal digesta was noted for birds fed all 4 enzyme-supplemented diets. It would appear from this study that the nutrient utilization of corn-SBM diet by broilers could be enhanced by using an appropriate multicarbohydrase enzyme supplement. The

nutrient encapsulating effect of cell wall polysaccharides in SBM, CM, and peas may not be the only factor responsible for incomplete nutrient utilization. The improvement in feed efficiency and starch availability in birds fed corn diet likely resulted from the cell wall degrading activity of the enzyme supplement.

4.2. Introduction

Soybean meal (SBM), canola meal (CM) and peas are the commonly used vegetable proteins in poultry diets in Canada. Their nutritive value, however, is limited by the presence of a number of antinutritive factors (ANF), including the indigestible nonstarch polysaccharides (NSP) (Slominski and Campbell, 1990; Bell, 1993; Castell et al., 1996; Dale, 1996). The major NSP components found in SBM, CM, and peas are pectic polysaccharides, which include rhamnogalacturonan with associated side chains consisting of arabinose, galactose, and xylose residues (Bacic et al., 1988). These sugars can occur in short or long, complex side chains containing neutral pectic polymers such as arabinans, galactans, or arabinogalactans (Aspinall and Cottrell, 1971; Siddiqui and Wood, 1972; Daveby and Aman, 1993). Other polysaccharides of SBM, CM, and peas include cellulose, xylans, arabinoxylans and xyloglucans, which are predominantly found in the hull fraction.

The successful use of enzymes in cereal-based diets (Chesson, 1993) has stimulated interest in the application of enzymes to target the vegetable protein components of poultry diets. A few studies reported to date have shown that targeting SBM, CM or peas with exogenous enzymes may be more challenging. Addition of a commercial enzyme product containing xylanase, amylase and protease activities to a

corn-SBM broiler diet results in a significant improvement in BW gain (1.9%) and feed-to-gain ratio (2.2%) as a result of increased ileal digestibility of protein and AME (Zanella, et al., 1999). By using the same enzyme product, however, Douglas et al. (2000) did not observe an improvement in growth performance of broilers fed a similar diet, although the ileal energy digestibility was significantly improved. In some other studies the inclusion of commercial enzyme complexes containing multicarbohydrase activities did not produce an improvement in growth performance of birds fed SBM-based diets, although a significant increase in protein and NSP digestibilities and AME content was observed (Marsman et al., 1997; Kocher et al. 2002). When using a carbohydrase cocktail of enzymes in a laying hen diet containing 40% CM, Slominski and Campbell (1990) demonstrated an increase in NSP digestibility. However, growth performance was not affected when similar enzyme products were added to broiler diets with high level of CM (Simbaya et al., 1996; Kocher et al, 2000; 2001). Brenes et al. (1993a) reported that enzyme supplementation improved feed-to-gain ratio of broiler chickens fed high-tannin but not low-tannin pea dies. Igbasan and Guenter (1996) demonstrated that pectinase supplementation of pea-based broiler diets improved BW gain and feed intake. However, in other studies the addition of pectinase preparations to pea-based diets did not result in improved chick performance (Igbasan et al., 1997b; Daveby et al., 1998).

Despite the inconsistency of the above findings, enzyme application in diets based on SBM, CM or peas seem to produce some beneficial effects in nutrient utilization and growth performance of broilers. Furthermore, in most of the studies, a significant increase in NSP digestibility was observed, indicating that enzymatic degradation of cell

wall polysaccharides is possible despite the complex nature of these polymers. In a recent study from our laboratory (Manuscript 1), a multicarbohydrase supplement containing several different enzyme activities was effective in depolymerizing cell wall polysaccharides of SBM, CM, and peas in vitro. The addition of the same enzyme supplement to a broiler diet based on wheat, SBM, CM, and peas resulted in a significant improvement in digestibility of protein, starch, and NSP and, consequently, improved growth performance. However, it was difficult to establish a clear enzyme-substrate relationship when using such a mixed diet.

When evaluating the enzyme effects on vegetable proteins, corn has been used extensively as a cereal component of poultry diets. Corn cell wall composition is similar to that of wheat (Chesson, 2001). Although branched arabinoxylans predominate in the endosperm cell walls, small amounts of mixed-linked β-glucan and cellulose are also present. The cell walls of the hull fraction are rich in xylans and cellulose. There is strong evidence that some nutrients in corn are not completely digested in the small intestine, and that considerable amounts of starch and protein escape digestion, reach the hindgut and undergo fermentation with a relatively low energy yield (Noy and Sklan, 1995). This has been partially attributed to the physical barrier created by the aleurone layer and starchy endosperm cell walls, which limit the animals' own digestive enzymes in accessing and fully digesting the starch and protein components enclosed within the cells (Theander et al., 1989; Slominski et al., 1993; Bedford, 2002). This highlights the opportunity for the use of feed enzymes in corn-based diets. However, in most of the studies to date corn has been considered to lack the response from enzyme supplementation. Therefore, it would appear important to evaluate the response of corn to

enzyme supplementation in order to distinguish the effects of enzyme addition on dietary components other than corn.

The objective of this study was to investigate the effect of a multicarbohydrase supplement of cell wall degrading enzymes on the nutritive value of SBM, CM or peas when fed to broiler chickens. The response of a semi-purified corn diet to enzyme addition was also investigated.

4.3. Materials and Methods

4.3.1. Experimental Diets

Four diets used in the study (Table 12) included a semipurified corn diet and 3 diets each containing 30% of either SBM, CM, or peas in addition to corn. Casein, fishmeal, and soy protein isolate were added to each diet to allow the targeted ingredients to serve as the only source of NSP. Each diet was mixed without and with an enzyme supplement (added at the expense of corn). The enzyme preparation was a multicarbohydrase cocktail of cell wall degrading activities and provided 1,000 U xylanase, 400 U glucanase, 1,000 U pectinase, 120 U cellulase, 280 U mannanase, and 180 U galactanase per kilogram of diet. The activity units of enzymes were defined in Manuscript 1. Enzyme supplements were provided by Canadian Bio-Systems Inc.[1]. The diets were isoenergetic and isonitrogenous and were formulated to be lower in AME (95%) and crude protein (95%) than the NRC (1994) specification in order to make the diets more sensitive to enzyme effects. Other nutrients met or exceeded NRC

[1] Calgary, Alberta, Canada.

TABLE 12. Composition and calculated analysis of the basal diets (g/kg)

	Corn diet	Corn-soybean meal diet	Corn-canola meal diet	Corn-peas diet
Ingredient				
Corn (8.5% CP)	687.5	585.0	542.0	516.0
Soybean meal (43.6% CP)		300.0		
Canola meal (36% CP)			300.0	
Peas (22.3% CP)				300.0
Casein(83% CP)	50.0	20.0	30.0	45.0
Fish meal (64.2% CP)	70.0	5.0	20.0	40.0
Soy protein isolate (84.0%CP)	74.0	10.0	19.0	40.0
Canola oil	15.0	30.0	46.0	14.5
Limestone[1]	14.0	14.0	12.5	16.5
Dicalcium phosphate[2]	9.0	16.4	11.5	7.5
DL-methionine	2.5	1.6	0.6	2.5
L-lysine-HCl			0.4	
Mineral premix[3]	5.0	5.0	5.0	5.0
Vitamin premix[4]	10.0	10.0	10.0	10.0
Chromic oxide	3.0	3.0	3.0	3.0
Silica sand	60.0			
Total	1,000.0	1,000.0	1,000.0	1,000.0
Calculated analysis				
CP (%)[5]	20.9	21.0	21.0	21.0
AME (kcal/kg)	3,055.0	3,050.0	3,050.0	3,050.0
Lysine (%)	1.21	1.20	1.20	1.20
Methionine (%)	0.65	0.59	0.59	0.65
Methionine + cystine (%)	0.91	0.90	0.92	0.89
Calcium (%)	1.03	1.00	1.00	1.00
Available phosphorous (%)	0.45	0.45	0.45	0.45

[1]Contained 38% calcium.

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

[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 of feed ingredients.

specifications for broiler chickens. Each diet contained chromic oxide (3 g/kg) as a marker for the calculation of nutrient digestibility coefficients and AME_n content.

4.3.2. Bird Management and Sample Collections

One-day-old male Arbor Acres broiler chicks were obtained from a local commercial hatchery. The birds were held in electrically heated battery brooders[2] for a 4-d pre-experimental period and were fed commercial chick starter crumbles (21% protein). On d 5, birds were fasted for 4 h, individually weighed, and sorted into 5 weight classes. Groups of 5 birds were then randomly assigned to pens such that the average initial BW of birds was similar across pens. Nine replicate pens of 5 birds each were randomly assigned to the 8 dietary treatments. All diets were fed in a mash form throughout the 2-wk experimental period. The birds had free access to water and feed and were provided with continuous light. Body weight and feed intake were monitored weekly using pens as the experimental units. Before weighing, the birds were fasted for 4 h. Mean BW, feed intake and feed-to-gain ratio were used to determine growth performance.

Excreta samples from each pen were collected on d 18 over a 3-h period and subsequently frozen, freeze-dried, and finely ground. The samples were analyzed for chromic oxide, gross energy, nitrogen, NSP, and starch contents. The total tract digestibility of starch and NSP and AME_n content of the experimental diets were calculated. On d 19 and 20, 20 birds from each treatment were randomly selected and killed by cervical dislocation. The contents of the ileum (from Meckel's diverticulum to 1cm above the ileo-cecal junction) were collected. The digesta samples were frozen, freeze-dried, ground, and pooled (digesta samples from 4 birds) to yield 5 replicate

[2] Jamesway battery brooders, James Mfg. Co., Mount Joy, PA.

samples per treatment. The samples were analyzed for chromic oxide, nitrogen, NSP, and starch to determine ileal digestibilities. The water-soluble and water-insoluble NSP were also measured in the ileal digesta samples.

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

4.3.3. Chemical Analysis

Diet, digesta, and excreta samples were analyzed in duplicate. Chromic oxide was determined using the procedure described by Williams et al. (1962). Nitrogen content was analyzed by the combustion method with a LECO Model FP 2000 combustion analyzer[3] and the protein contents were calculated using the multiplication factor of 6.25. Gross energy was determined using a Parr 1261 adiabatic bomb calorimeter[4]. Starch was determined colorimetrically using Sigma Glucose (HK) 20 kit and the procedure described by Aman and Hasselman (1984).

The NSP was determined by gas-liquid chromatography (component neutral sugars) and by colorimetry (uronic acids). The procedure for neutral sugars was as described by Englyst and Cummings (1984, 1988) with some modifications (Slominski and Campbell, 1990). Briefly, 100 mg diet samples or 50 mg digesta or excreta samples were boiled with 2mL dimethylsulfoxide for 1 h and then incubated at 45°C overnight with a sodium acetate buffer solution (pH 5.2) of starch-degrading enzymes (amylase, pullulanase, and amyloglucosidase[5]). Ethanol was then added and the mixture was left for

[3] LECO Corp., St. Joseph, MI.
[4] Parr Instrument Co., Moline, IL.
[5] Sigma, St. Louis, MO.

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[6]. Uronic acids were determined using the procedure described by Scott (1979). Water-soluble NSP contents of diets and digesta samples was determined according to the method described by Slominski et al. (1993). The water-insoluble NSP content in ileal digesta was calculated as the difference between total NSP and water-soluble NSP content. The proportion of water-soluble NSP in total NSP was calculated for each diet.

4.3.4. Calculations and Statistical Analysis

The following equations were used to calculate the digestibility of various dietary components (using starch digestibility calculation as an example) and AME_n content of experimental diets (Hill et al., 1960):

$$\text{Digestibility (\%)} = \{1 - [(Cr_2O_3\ _{\%\ \text{diet}} / Cr_2O_3\ _{\%\ \text{digesta/ excreta}}) \times (\text{Starch}\ _{\%\ \text{digesta/ excreta}} / \text{Starch}\ _{\%\ \text{diet}})]\} \times 100,$$

$$AME_n\ (\text{kcal/kg}) = GE\ _{\text{kcal/kg diet}} - [GE\ _{\text{kcal/kg excreta}} \times (Cr_2O_3\ _{\%\ \text{diet}} / Cr_2O_3\ _{\%\ \text{excreta}})] - 8.22 \times \{N\ _{\%\ \text{diet}} - [N\ _{\%\ \text{excreta}} \times (Cr_2O_3\ _{\%\ \text{diet}} / Cr_2O_3\ _{\%\ \text{excreta}})]\}$$

[6] Varian Canada Inc., Mississauga, Ontario.

Where GE is gross energy, N is nitrogen, Cr_2O_3 is chromic oxide, and 8.22 is the energy equivalent of uric acid nitrogen (i.e., 8.22 kcal/kg uric acid nitrogen).

The experiment was set up as a completely randomized design. All data were subjected to two-way ANOVA using the GLM Procedure of SAS (SAS Institute, 1986) to determine the effect of enzyme addition, diet type, and interactions between diet type and enzyme addition. Linear contrasts of enzyme effects (i.e., with vs. without enzyme addition) within each diet type were made within the analysis of variance and used for comparison of treatment means. As it was not the objective of this study to compare various variables among diets; results of the effect of diet were presented but not discussed in this paper.

4.4. Results

4.4.1. Total and Water-Soluble NSP Contents of Feed Ingredients and Experimental Diets

Total and water-soluble NSP in corn, SBM, CM, and peas and in basal diets are presented in Table 13. The total NSP content of corn was much lower than that of SBM, CM, and peas. However, the water-soluble NSP of corn accounted for 8.4% of its total NSP content and was similar to those of SBM and CM but higher than that of peas (4.7%). Although the corn diet was still lower in total NSP, the difference in total NSP content among diets was much less than that observed for the individual ingredients. The percentage of water-soluble NSP in total NSP content was found to range from 8.5 to 9.3% for corn, SBM, and CM diets and was lower (6.7%) for the pea diet.

TABLE 13. Total and water-soluble nonstarch polysaccharides (NSP) contents of feed ingredients and experimental diets

Item	Total NSP (mg/g)	Water-soluble NSP (mg/g)	Water-soluble NSP (% of total NSP)
Ingredient			
Corn	76.3	6.4	8.4
Soybean meal	136.7	13.4	9.8
Canola meal	174.5	14.3	8.2
Peas	124.7	5.9	4.7
Diet			
Corn diet	51.0	4.3	8.5
Corn-soybean meal diet	90.1	8.4	9.3
Corn-canola meal diet	95.1	8.4	8.8
Corn-peas diet	79.1	5.3	6.7

4.4.2. Growth Performance

The growth performance of broilers during the 2-wk experimental period is presented in Table 14. Enzyme supplementation had no effect ($P > 0.05$) on feed intake and BW gain but affected ($P < 0.05$) feed-to-gain ratio. A comparison of the data using linear contrasts demonstrated that enzyme addition to the corn diet tended ($P = 0.054$) to improve BW gain and significantly improved ($P < 0.05$) feed-to-gain ratio. However, only a trend in improved BW gain ($P = 0.07$) and feed-to-gain ratio ($P = 0.08$) was observed following inclusion of enzymes in the corn-SBM diet. The performance of birds fed the CM or pea diet was not affected ($P > 0.05$) by enzyme supplementation. There was a significant interaction ($P < 0.05$) for feed-to-gain ratio between diet type and enzyme addition, which was the result of a significant enzyme effect on the corn diet, whereas there was no enzyme effect for the other diets.

4.4.3. Total Tract Digestibility of NSP and Starch and AME_n Content

Total tract digestibility of NSP and starch and AME_n content of experimental diets is summarized in Table 15. Enzyme supplementation had a significant effect ($P < 0.001$) on total tract digestibility of NSP and AME_n value but did not affect ($P > 0.05$) the total tract digestibility of starch. Although an improvement ($P < 0.05$) in total tract digestibility of NSP was observed for all enzyme-supplemented diets, the increase ($P < 0.05$) in AME_n content was observed only for the corn and corn-SBM diets. The significant interaction ($P < 0.05$) between diet type and enzyme addition indicated that the enzyme effect on AME_n differed depending on the diet type.

TABLE 14. Growth performance of broiler chickens (5 to 18 d) fed diets without (–) or with (+) multicarbohydrase enzyme supplementation[1]

Diet type	Feed intake (g/bird)	Body weight gain (g/bird)	Feed / gain
Corn diet			
–	601[2]	430	1.400
+	619	455	1.360
P	0.213	0.054	0.025
Corn-soybean meal diet			
–	637	473	1.347
+	653	496	1.316
P	0.252	0.072	0.084
Corn-canola meal diet			
–	576	399	1.443
+	570	393	1.448
P	0.668	0.649	0.742
Corn-peas diet			
–	519	367	1.416
+	536	377	1.423
P	0.244	0.433	0.688
Pooled SEM	10.0	8.9	0.0119
Source of variation		Probability	
Diet type	<0.001	<0.001	<0.001
Enzyme	0.215	0.126	0.011
Diet type × enzyme	0.669	0.208	0.015

[1]A multicarbohydrase supplement containing cellulase, pectinase, xylanase, glucanase, mannanase and galactanase as main activities.

[2]Means of 9 replicate pens of 5 birds each.

TABLE 15. Apparent total tract nonstarch polysaccharides (NSP) and starch digestibilities, and AME_n content of diets fed to broilers without (–) or with (+) multicarbohydrase enzyme supplementation[1]

Diet type	NSP (%)	Starch (%)	AME_n (kcal/kg diet; as fed basis)
Corn diet			
–	8.2[2]	97.6	3209
+	13.4	98.2	3315
P	0.010	0.251	<0.001
Corn-soybean meal diet			
–	9.4	96.0	3114
+	21.1	97.2	3186
P	<0.001	0.201	<0.003
Corn-canola meal diet			
–	7.6	96.0	3042
+	16.9	96.3	3086
P	<0.001	0.608	0.096
Corn-peas diet			
–	4.5	91.6	3109
+	9.5	92.9	3149
P	0.012	0.109	0.139
Pooled SEM	1.34	0.56	18.5
Source of variation		Probability	
Diet type	<0.001	<0.001	<0.001
Enzyme	<0.001	0.300	<0.001
Diet type × enzyme	0.112	0.439	0.031

[1]A multicarbohydrase supplement containing cellulase, pectinase, xylanase, glucanase, mannanase and galactanase as main activities.

[2]Means of 5 pooled samples of 4 birds each.

4.4.4. Ileal Digestibility of Starch and Protein and NSP Concentrations in Ileal Digesta

Ileal digestibility of starch and protein, and the contents of water-soluble and -insoluble NSP of the ileal digesta are shown in Table 16. The addition of enzymes had a significant effect ($P < 0.001$) on ileal starch digestibility and ileal water-soluble and water-insoluble NSP concentrations but not on ileal protein digestibility ($P > 0.05$). Contrast analysis showed that ileal starch digestibility was improved significantly ($P < 0.05$) with enzyme addition only for the corn diet. Therefore, a significant interaction between diet type and enzyme addition was recorded for this parameter. Protein digestibility was improved ($P < 0.05$) by enzyme addition to the corn-SBM diet but decreased for the corn-CM diet, which resulted in a significant interaction ($P < 0.05$) between diet type and enzyme addition. The ileal concentration of water-soluble NSP increased ($P < 0.05$) and water-insoluble NSP decreased ($P < 0.05$) following enzyme addition to all the diets.

4.5. Discussion

The total NSP contents of SBM, CM, and peas are in good agreement with the values of 148, 171, and 127 mg/g previously determined in our laboratory for SBM, CM, and peas, respectively (Manuscript 1). The proportions of water-soluble NSP in total NSP content measured in the current study were similar to those reported in literature. According to Cowan et al. (1996), water-soluble NSP accounts for 7.2, 8.0, and 4.7% of the total NSP content of corn, SBM, and peas, respectively. Similar values of 8.4% (Bell, 1993) and 9.8% (Kocher et al., 2000) were reported for CM. In corn, a water-soluble fraction of NSP accounts for 9.3% of the total NSP content (Bach

TABLE 16. Apparent ileal starch and protein digestibilities, and water-soluble and water-insoluble nonstarch polysaccharide (NSP) contents of ileal digesta from broilers fed diets without (–) or with (+) multicarbohydrase enzyme supplementation[1]

Diet type	Starch (%)	Protein (%)	Water-soluble NSP (mg/g)	Water-insoluble NSP (mg/g)
Corn diet				
–	92.7[2]	72.7	14.1	209
+	95.3	71.8	17.4	182
P	0.001	0.609	0.045	0.036
Corn-soybean meal diet				
–	93.2	79.6	23.8	272
+	95.2	84.0	29.6	244
P	0.101	0.016	0.001	0.022
Corn-canola meal diet				
–	93.4	76.9	25.3	281
+	94.8	73.5	30.0	255
P	0. 304	0.043	0.008	0.045
Corn-peas diet				
–	87.3	65.8	17.6	263
+	89.5	67.8	21.8	238
P	0.107	0.253	0.019	0.048
Pooled SEM	0.85	1.10	1.19	8.4
Source of variation	Probability			
Diet type	<0.001	<0.001	<0.001	<0.001
Enzyme	0.030	0.603	<0.001	<0.042
Diet type × enzyme	0.032	0.031	0.503	0.629

[1]A multicarbohydrase supplement containing cellulase, pectinase, xylanase, glucanase, mannanase and galactanase as main activities.

[2]Means of 5 pooled samples of 4 birds each.

Knudsen, 1997). Evidently, corn, SBM, CM, and peas contain less water-soluble NSP than wheat, for which values of 14.7% (Cowan et al., 1996) and 23.8% (Steenfeldt, 2001) have been reported. It appears that the lower level of soluble NSP in corn, SBM, CM, or peas may contribute to the lower efficacy of feed enzymes in diets based on these feedstuffs. In this regard, the water-soluble NSP would be considered more susceptible to enzyme action especially under a short digesta transit time in the GI tract (Danicke et al., 1999a).

The present study demonstrated that addition of the multicarbohydrase supplement of cell wall degrading enzymes improved feed-to-gain ratio in broiler chickens fed the diet containing corn as the only source of NSP. To our knowledge, there are no comparable published data to illustrate such an effect. Nutrient digestibility values indicated that the improvement in feed-to-gain ratio resulted from increased ileal starch digestibility, suggesting that the added enzymes improved starch availability to support more efficient chick growth. The significant increase in water-soluble and decrease in water-insoluble NSP in ileal digesta of birds fed the enzyme-supplemented corn diet further documented that the beneficial effects of the supplemented enzymes could be due to the partial breakdown of endosperm cell walls thereby exposing starch to rapid digestion by the birds' intestinal amylases.

Starch is the major energy source in corn. The significant improvement in ileal starch digestibility could have contributed to the increase in available energy (AME_n) content of the corn diet. It can be concluded that the nutritive value of corn can be improved by NSP-degrading enzymes. These findings also indicate that when using corn as a base feed ingredient in enzyme research to target various other dietary components,

some beneficial effects of enzyme addition could originate from corn when the enzyme supplements contain activities toward corn NSP.

In agreement with earlier research (Noy and Sklan, 1995; Zanella et al., 1999), the present study demonstrated that starch digestibility, when measured by excreta collection, was high in birds fed corn diet but was lower when determined at the ileal level. This finding confirms that starch digestion is incomplete in the small intestine and is completed in the hindgut as a result of microbial fermentation (Persia and Lilburn, 1998). Therefore, the hindgut fermentation may contribute to the lack of difference in starch digestibility between the non-enzyme- and enzyme-supplemented corn diets when measured at the excreta level. Hence, it may be concluded that measuring starch digestibility at the ileal level may better reflect the response from enzyme addition. It has been suggested that in addition to starch, considerable amounts of corn protein could be shielded by the cell walls and, thus, escape digestion (Pack et al. 1998). In the current study, however, ileal protein digestibility was not affected by enzyme addition to the corn diet. A small contribution of corn protein to the overall protein content of the diet may explain the lack of response.

Enzyme addition to the corn-SBM diet did not affect chick performance significantly, although NSP and protein digestibilities were improved, and there was a trend ($P = 0.1$) towards improved ileal starch digestibility with enzyme supplementation. Similar results and the lack of improvement in growth performance of broilers fed similar corn-SBM diets have been reported by other authors (Marquardt et al., 1994; Marsman et al., 1997; Douglas et al., 2000; Kocher et al., 2002). However, Zanella et al. (1999) demonstrated a small but significant performance improvement (1.9-2.2%) associated

with enzyme addition when using a similar diet. These findings, together with a trend in performance improvements observed in the current study for the enzyme-supplemented corn-SBM diet may suggest that feed enzymes based on cell wall degrading activities do exert some beneficial effects on broiler chicken performance when fed corn-SBM diets, although such effects may be small and difficult to detect in a small-scale experiment. However, such improvements may be of economical importance for large-scale broiler operations. Furthermore, the use of other enzyme activities (i.e., amylases, proteases) in addition to cell wall degrading enzymes may be even more effective. In this regard, earlier research from our laboratory demonstrated that the use of multienzyme preparations in corn-SBM diets resulted in a consistent improvement in broiler chicken performance with the improvements in BW gain averaging 4.0% and feed-to-gain ratio averaging 2.5% (Slominski, 2000). However, as was the case in the current study, of 3 similar 2-wk growth performance experiments with broiler chickens, the statistically significant ($P < 0.05$) difference was only observed in 1 experiment for BW gain and in 2 experiments for feed-to-gain ratio.

It is of interest to note that the improvement in AME_n content of enzyme-supplemented corn-SBM diet was the result of improved protein digestibility, and only a trend ($P < 0.1$) in improved starch digestibility was noted. The reason that the enzyme supplement improved ileal digestibility of starch for the corn diet but not for the other diets containing the same batch of corn is not clear. One possible explanation could be that the inclusion rate of corn in the corn diet was much higher than that in the other diets (69% vs 52-59%). In this regard, it has been documented that the enzyme effect

decreased with decreased inclusion rate of wheat in the wheat-based diets (Steenfeldt et al., 1998).

As indicated earlier, enzyme addition to the corn diet resulted in no improvement in ileal protein digestibility. Therefore, the significant improvement in protein digestibility observed for birds fed the corn-SBM diet could mainly result from the effect of enzymes on cell wall polysaccharides of SBM as illustrated by the higher NSP digestibility values for the enzyme-supplemented diet. Prepress-solvent extraction is currently the most effective method of extracting oil from oilseeds (Newkirk et al., 2003). In this regard, the flaking and expelling processes used during soybean processing largely rupture the cell wall structure as evidenced by high oil yield and less than 1% residual oil content in the final meal. This would indicate that very little intact cell wall material is present in SBM. Therefore, the significant increase in ileal protein digestibility observed in the current study for the enzyme-supplemented diet may have resulted more from the disruption of the cell wall matrix of SBM, leading to the release of structural protein (i.e., glycoprotein), rather than from the elimination of encapsulating effect of the cell walls.

The present study demonstrated that the use of an enzyme preparation with cell wall degrading activities to improve the nutritive value of CM and peas for broilers was not effective. Carbohydrase enzyme addition did not influence the growth performance, starch digestibility or AME_n content of CM and pea diets. Similar results were reported earlier for CM-based diets (Simbaya et al., 1996; Kocher et al., 2000) and pea-based diets (Brenes et al., 1993a; Igbasan et al., 1997b). Although in the current study, the NSP of CM or peas were partially degraded as evidenced by the improved NSP digestibilities following enzyme addition, birds did not benefit from this effect. It has been suggested

by Moran (1982) that poultry are not able to produce and use any significant amount of energy deriving from volatile fatty acid production. Therefore, a limited use of the NSP hydrolysis products may explain the lack of enzyme effects on AME_n content and chick performance when fed CM or pea diet. As is the case for soybeans, the flaking, cooking, and mechanical pressing used in canola seed processing may also explain the lack of improvement in protein digestibility by birds fed the corn-CM diet supplemented with cell wall degrading enzymes. In fact, the present study showed that the inclusion of the multicarbohydrase supplement in the CM diet tended to decrease ileal protein digestibility. Kocher et al. (2002) also found a reduction in ileal nitrogen retention and protein digestibility when a galactanase enzyme was added to a corn-SBM diet. The reason for this is not clear, but it may indicate that the enzyme effects may not always be beneficial, and some low-molecular weight NSP hydrolysis products may adversely affect protein digestion by chickens (Irish and Balnave, 1993; Acamovic, 2001).

In addition to protein, feed peas contain up to 45% starch in their cotyledons (Castell et al., 1996). Using microscopical examination, Wursch et al. (1986) demonstrated that pea starch, together with associated protein is located in parenchyma cells of thick walls which may limit starch digestibility. However, in the present study, the improved NSP digestibility did not result in a significant increase in the digestibility of starch and protein when enzyme was added to the corn-pea diet. It appears that nutrient encapsulating effect of the cell walls may not be responsible for incomplete nutrient utilization by broilers fed a pea diet. When compared with cereal grain starches, pea starch has a higher amylose-to-amylopectin ratio. According to Daveby et al. (1998), amylose is relatively less digestible by nonruminants than amylopectin. Also, in pea

starch a C pattern granule predominates, which is more resistant to pancreatic amylase than the A pattern typical for cereal grains (Canibe and Bach Knudsen, 1997; Daveby et al., 1998). Therefore, when compared with the other diets, the relatively lower starch digestibility of the pea diet measured at either ileal or fecal level may reflect the resistant nature of pea starch to digestion by broilers.

The present study demonstrated that addition of carbohydrase enzymes improved NSP digestibility for all the diets. It is of interest to note that in non-enzyme supplemented diets, NSP were digested to a degree corresponding well with the water-soluble NSP fraction of the diets (i.e., 8.2 vs 8.4%, 9.4 vs 9.8%, 7.6 vs 8.2%, and 4.5 vs 4.7% for the corn, SBM, CM, and pea diets, respectively). This finding indicates that in the absence of enzyme supplement, only water-soluble NSP were digested. This is in agreement with the observation by Carre et al. (1990, 1995), who found that in the absence of enzyme supplement, water-soluble NSP are the only fraction that can be digested by poultry, whereas the water-insoluble NSP remain almost undigested. However, with enzyme supplementation NSP were digested to a degree far beyond the level of soluble NSP portion of each diet (i.e., 13.4 vs 8.4%, 21.1 vs 9.8%, 16.9 vs 8.2%, and 9.5 vs 4.7% for corn, SBM, CM, and pea diets, respectively). This result suggests that the multicarbohydrase enzyme supplement used in the present study not only hydrolyzed water-soluble NSP, but also partially depolymerized the water-insoluble NSP fraction of the feed ingredients. This was illustrated by the significant increase in water-soluble and decrease in water-insoluble NSP concentrations in the ileal digesta of birds fed enzyme-supplemented diets. The enzyme supplement used in the current study contained a number of cell wall degrading activities including pectinase, cellulase,

galactanase, xylanase, glucanase, and mannanase. Similar to results from our earlier in vitro work (Manuscript 1), these enzymes may have acted in a coordinated manner to enhance the degradation of cell wall polysaccharides of SBM, CM, and peas in vivo. However, only birds fed the corn-SBM diet benefited from the improved NSP digestibility in terms of protein digestibility, AME_n content, and to some extent growth performance. It may be, therefore, speculated that the significant improvements in growth performance and nutrient digestibility observed in our previous research in which the same enzyme preparation was supplemented to a wheat-SBM-CM-peas-based diet (Manuscript 1) resulted from the enzyme effects on wheat and, possibly, SBM rather than on CM and peas.

In conclusion, it would appear from this study that the nutrient utilization of a corn-SBM diet by broilers could be enhanced by a multicarbohydrase supplement of cell wall degrading activities. The failure to detect any improvement in nutritive value of CM and peas by carbohydrase enzymes indicates that the nutrient encapsulating effects of cell wall polysaccharides in CM and peas may not be a factor responsible for less than optimum nutrient utilization by broiler chickens. The lack of relationship between NSP digestibility and AME_n content of the CM or pea diet may reflect a limited contribution of NSP hydrolysis products to available energy content via the process of lower gut fermentation. The improvement in feed efficiency in birds fed the enzyme-supplemented corn diet was associated with the increase in starch availability due to cell wall degrading activities of the enzyme supplement.

4.6. Acknowledgements

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5. MANUSCRIPT 3

The Effect of Dietary Fat Type, Carbohydrase, and Lipase Addition on Growth Performance and Nutrient Utilization of Young Broilers Fed Wheat-Based Diets

5.1. Abstract

A 2 × 2 × 2 factorial experiment was conducted to evaluate the effects of fat type [beef tallow (50 g/kg diet) or canola oil (50 g/kg diet)], carbohydrase addition [none or carbohydrases (0.4 g/kg diet)] and lipase addition [none or lipase (0.2 g/kg diet)] on growth performance and nutrient utilization of male broilers fed a wheat-based diet from 5 to 18 d. The carbohydrase supplement contained xylanase, glucanase, cellulase and other enzyme activities. The experimental diets were formulated to be suboptimal in major nutrients and each was fed in a mash form to 10 replicate pens of 5 broilers per pen. Body weight gain was not affected by fat type but a poorer feed-to-gain ratio ($P < 0.001$) was noted for tallow-containing diets. Regardless of fat type, carbohydrase enzyme supplementation improved ($P < 0.001$) BW gain and feed-to-gain ratio. There was no effect of lipase addition on chicken performance and nutrient utilization. When compared with canola oil, tallow-containing diets had a lower ($P < 0.001$) apparent fat digestibility and consequently a lower diet AME_n content. Carbohydrase enzyme addition improved ($P < 0.001$) fat, starch, nitrogen and nonstarch polysaccharide (NSP) digestibilities in the small intestine, improved AME_n, and reduced ($P < 0.001$) jejunal digesta viscosity in both fat types. Carbohydrase supplementation increased water-soluble ($P < 0.001$) and decreased water-insoluble ($P < 0.001$) NSP concentrations in the small intestine. The interaction between fat type and carbohydrase addition was only significant for fat digestibilities, with greater improvements seen for diets containing tallow. Significant interactions between carbohydrase addition and intestinal segment were noted for fat, starch, nitrogen and NSP digestibilities, with the enzyme effects being greater in the jejunum than the ileum. It is evident from the present study that an appropriate

carbohydrase preparation could eliminate the negative effects of soluble NSP on animal fat utilization in a wheat-based broiler diet.

5.2. Introduction

Animal fats and vegetable oils are usually added to broiler diets to increase energy concentration and to improve productivity. The digestibility of a dietary fat depends on the chemical nature of its constituent fatty acids (Garrett and Young, 1975; Ketels and De Groote, 1989; Danicke et al., 2000). Fats rich in unsaturated fatty acids are better digested and absorbed than saturated fats (Danicke, 2001). Beef tallow is characterized by a lower fat digestibility and a lower ME content than vegetable oils, and these have been attributed to its higher content of long-chain saturated fatty acids (LCSFA) (Blanch et al., 1995).

Water-soluble fractions of nonstarch polysaccharides (NSP), including arabinoxylan of rye and wheat and β-glucan of barley, are known to exert adverse effects on the performance and nutrient digestibility in broilers (Bedford and Classen, 1992; Choct and Annison, 1992a). Such negative effects are thought to be caused by an increased digesta viscosity and can be largely eliminated by the addition of viscosity-reducing carbohydrase enzymes such as xylanase and β-glucanase (Choct and Annison, 1992b, Simon, 1998; Steenfeldt et al., 1998a, b). Several studies have shown that, among the nutrients, fat digestion suffers the most pronounced impairment due to high digesta viscosity (Campbell et al., 1983; Ward and Marquardt, 1983; Choct and Annison, 1992b). Furthermore, an increase in intestinal viscosity is more detrimental to the digestion and absorption of dietary fats containing high proportions of saturated fatty acids. Antoniou et

al. (1980) observed a greater depression in performance and fat digestibility in rye fed birds when tallow rather than soya oil was added to the diet. Using similar rye-based broiler diets, Danicke et al. (1997, 1999b, 2000) demonstrated that dietary fat type influenced the degree of the carbohydrase enzyme effects on fat digestion.

Compared with rye, wheat contains a relatively lower level of arabinoxylan and thus produces lower viscosity (Henry, 1987). However, Preston et al. (2001) demonstrated a significant interaction between fat type and enzyme effect in a wheat-based (70%) broiler diet. Furthermore, Pasquier et al. (1996) reported reduced fat emulsification and hydrolysis with every increment in medium viscosity over a range from 0 to 20 mPa s. These results suggest that the negative effect of viscous NSP on fat digestion and absorption may not be confined to diets that induce high intestinal viscosity.

It is well known that fat digestion is facilitated by the combined action of bile acids, lipase and co-lipase. It has been demonstrated that the physiological functions necessary for efficient fat digestion in young chickens are immature and continue to develop for several weeks after hatching (Jin et al., 1998). Noy and Sklan (1995) reported that in broiler chickens, secretion of lipase was low at hatching and increased 20-fold between 4 and 21 d of age. Krogdahl and Sell (1989) also reported that dietary tallow and animal-vegetable fat were not efficiently used until the lipase activity reached its maximum level. Because young birds have insufficient secretion of endogenous lipase, dietary supplementation of bacterial lipase may improve fat use.

In many studies fat type × enzyme interactions were evaluated using high fat inclusion rates (e.g. 10%) and highly viscous semisynthetic diets (e.g. rye-based), but

such information regarding practical wheat-based broiler diets is limited. Therefore, the goal of the present study was to investigate the growth performance and nutrient utilization responses of broilers to the dietary fat type, carbohydrase, and lipase supplementation using a wheat-based diet.

5.3. Materials and Methods

5.3.1. Experimental Design and Diets

A 2 × 2 × 2 factorial experiment was used to evaluate the effects of dietary fat type [beef tallow (50g/kg diet) or canola oil (50g/kg diet)], carbohydrase addition [none or carbohydrases (0.4 g/kg diet)] and lipase addition [none or lipase (0.2 g/kg diet)] to a wheat (60%)-based broiler diet. The 2 basal diets (Table 17) containing beef tallow or crude canola oil were formulated to meet 95% of the NRC (1994) requirement for AME and 92% of CP, calcium, available phosphorus, methionine, and methionine + cysteine. Other nutrients met or exceeded NRC specifications. Beef tallow (melting point, 43°C; saturated fatty acids, 49.5%; monounsaturated fatty acids 42.0%; and polyunsaturated fatty acids, 3.0% of the total fat) and crude canola oil (melting point, -10°C; saturated fatty acids, 6.0%; monounsaturated fatty acids 55.0%; and polyunsaturated fatty acids, 36.0% of the total fat) were provided by a local feed manufacturer. The carbohydrase enzyme preparation was a multicarbohydrase cocktail and supplied xylanase (1000 units/kg diet), glucanase (400 units/kg diet), cellulase (120 units/kg diet) and other NSP-degrading activities that were determined in this laboratory (Manuscript 1). In addition to wheat, the carbohydrase preparation was effective in soybean meal, canola meal and pea

TABLE 17. The composition and chemical analysis of basal diets (g/kg)

	Canola oil diet	Beef tallow diet
Ingredient		
Wheat (13.5% CP)	600.0	600.0
Soybean meal (46% CP)	215.0	215.0
Canola meal (36% CP)	60.0	60.0
Peas (22% CP)	30.0	30.0
Canola oil	50.0	
Tallow		50.0
Limestone[1]	14.6	14.6
Dicalcium phosphate[2]	10.8	10.8
DL-methionine	0.8	0.8
L-lysine-HCl	0.8	0.8
Mineral premix[3]	5.0	5.0
Vitamin premix[4]	10.0	10.0
Chromic oxide	3.0	3.0
Total	1,000.0	1,000.0
Calculated analysis		
Crude protein (%)[5]	21.0	21.0
Crude fat (%)	7.8	7.8
AME (kcal/kg)	3,040.0	2,990.0
Lysine (%)	1.15	1.15
Methionine (%)	0.46	0.46
Methionine + cysteine (%)	0.86	0.86
Calcium (%)	0.92	0.92
Available Phosphorous (%)	0.41	0.41

[1]Contained 38% calcium.

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

[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 of ingredients.

NSP hydrolysis that, under the conditions of the assay (pH 5.2, 45°C), averaged 26, 36 and 28%, respectively. The lipase enzyme supplied 100 units of activity per kilogram of diet. Both enzyme supplements were provided by Canadian Bio-Systems Inc., Calgary, Canada. Chromic oxide at 3.0g/kg of diet was mixed with the diets and used to calculate the nutrient digestibilities and dietary AME_n content.

5.3.2. Growth Performance

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[1] for a 4-d pre-experimental period and fed commercial chick starter crumbles (21% CP). On d 5, birds were fasted for 4 h, individually weighed and sorted into 5 weight classes. Groups of five birds were then randomly assigned to pens such that the average initial BW of birds was similar across pens. Ten replicate pens of five birds each were randomly assigned to the 8 dietary treatments. All diets were fed in a mash form throughout the 2-wk experimental period. 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. Before weighing, the birds were fasted for 4 h. Mean BW, feed intake and feed-to-gain ratio were used to determine the performance of birds.

5.3.3. Nutrient Utilization

At the termination of the experiment (on d 18), excreta samples from each pen were collected over a 3 h period and subsequently frozen, freeze-dried, and finely ground. The samples were analyzed for chromic oxide, gross energy, nitrogen, NSP and fat

[1] James Mfg. Co., Mount Joy, PA

contents. The total tract digestibility of fat and NSP and AME_n content of experimental diets were calculated. On d 19 and 20, 20 birds from each treatment were randomly selected and killed by cervical dislocation. The contents of the jejunum (from the end of the duodenum to Meckel's diverticulum) and the ileum (from Meckel's diverticulum to 1cm above the ileo-cecal junction) were collected. The digesta samples were frozen, freeze-dried, ground and pooled to yield 5 replicate samples per treatment. The samples were analyzed for chromic oxide, nitrogen, NSP, starch, and fat to determine their jejunal and ileal digestibilities. The water-soluble and water-insoluble NSP concentrations were also measured for jejunal and ileal digesta samples.

In addition, 10 birds per treatment were randomly selected for intestinal viscosity measurement. The birds were killed by cervical dislocation and the contents of the jejunum were collected and pooled for 2 birds to yield 5 replicate samples per treatment. Fresh samples of 1.5 g each were centrifuged at 9,000 rpm for 10 min and the viscosity of the supernatant was determined at 40°C using the Brookfield digital viscometer[2].

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

5.3.4. Chemical Analysis

Diet, digesta, and excreta samples were analyzed in duplicate for fat content using the AOAC method 920.39 (AOAC, 1990). Chromic oxide was determined using the procedure described by Williams et al. (1962). Nitrogen content was analyzed by the

[2] Model DV-II+LV, Brookfield Engineering Laboratories, Stoughton, MA.

combustion method using the LECO Model FP 2000 combustion analyzer[3]. Gross energy was determined using a Parr 1261 adiabatic bomb calorimeter[4]. Starch was determined colorimetrically using a Sigma Glucose (HK) 20 kit and the procedure described by Aman and Hasselman (1984).

Total NSP were determined by gas-liquid chromatography (component neutral sugars) and by colorimetry (uronic acids) using the procedure described by Englyst and Cummings (1984, 1988) with minor modifications (Slominski and Campbell, 1990). In brief, 100 mg diet samples or 50 mg digesta or excreta samples were boiled with 2mL dimethylsulfoxide for 1 h and then incubated at 45°C overnight with a sodium acetate buffer solution (pH 5.2) of starch-degrading enzymes (amylase, pullulanase, and amyloglucosidase[5]). Ethanol was added 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 SP-2340 column and a Varian CP 3380 Gas Chromatograph[6]. Water-soluble NSP content of the digesta samples was determined according to the method described by Slominski et al. (1993). Water-insoluble NSP

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[3] LECO Corp., St. Joseph, MI.
[4] Parr Instrument Co., Moline, IL.
[5] Sigma, St. Louis, MO.
[6] Varian Canada Inc., Mississauga, Ontario.

content was calculated as the difference between total NSP and water-soluble NSP content.

5.3.5. Calculations and Statistical Analysis

The following equations were used for calculation of the digestibility of various dietary components (using fat calculation as an example) and AME_n content of experimental diets (Hill et al., 1960):

$$\text{Digestibility (\%)} = \{1 - [(Cr_2O_3\ \%\ \text{diet} / Cr_2O_3\ \%\ \text{digesta/ excreta}) \times (\text{Fat}\ \%\ \text{digesta/ excreta} / \text{Fat}\ \%\ \text{diet})]\} \times 100,$$

$$AME_n\ (\text{kcal/kg}) = GE_{\text{kcal/kg diet}} - [GE_{\text{kcal/kg excreta}} \times (Cr_2O_3\ \%\ \text{diet} / Cr_2O_3\ \%\ \text{excreta})] - 8.22 \times \{N\ \%\ \text{diet} - [N\ \%\ \text{excreta} \times (Cr_2O_3\ \%\ \text{diet} / Cr_2O_3\ \%\ \text{excreta})]\}$$

Where GE is gross energy, N is nitrogen, Cr_2O_3 is chromic oxide, and 8.22 is the energy equivalent of uric acid nitrogen, i.e. 8.22 kcal/kg uric acid nitrogen.

Three-way factorial ANOVA was applied for performance parameters, jejunal viscosity, AME_n, and total tract digestibility of fat and NSP:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + (A \times B)_{ij} + (A \times C)_{ik} + (B \times C)_{jk} + (A \times B \times C)_{ijk} + e_{ijkl}$$

Where Y_{ijkl} = tested parameter of a broiler l fed a diet containing fat type i, carbohydrase level j and lipase level k; A_i = fat type (beef tallow, canola oil); B_j = carbohydrase addition (none, carbohydrase 0.4 g/kg diet); C_k = lipase addition (none, lipase 0.2 g/kg

diet); $(A \times B)_{ij}$ = interactions between fat type and carbohydrase addition; $(A \times C)_{ik}$ = interactions between fat type and lipase addition; $(B \times C)_{jk}$ = interactions between carbohydrase and lipase addition; $(A \times B \times C)_{ijk}$ = interactions among fat type, carbohydrase, and lipase addition; e_{ijkl} = error term.

The digestibility of fat, starch, nitrogen, and NSP, and water-soluble and water-insoluble NSP concentrations in the jejunum and the ileum were analyzed by a 4-way ANOVA as a split-plot experiment with repeated measurements. Since the 3-way and 4-way interactions were tested non-significant, these components were removed from the complete model and the final model used was:

$$Y_{ijklm} = \mu + A_i + B_j + C_k + (A \times B)_{ij} + (A \times C)_{ik} + (B \times C)_{jk} + e_m(a \times b \times c) + D_l + (A \times D)_{il} + (B \times D)_{jl} + (C \times D)_{kl} + e_{ijklm}$$

Where Y_{ijklm} = tested parameter of a broiler m fed a diet containing fat type i, carbohydrase level j, lipase level k in intestinal segment l; A_i = fat type (beef tallow, canola oil); B_j = carbohydrase addition (none, carbohydrase 0.4 g/kg diet); C_k = lipase addition (none, lipase 0.2 g/kg diet); $(A \times B)_{ij}$ = interactions between fat type and carbohydrase addition; $(A \times C)_{ik}$ = interactions between fat type and lipase addition; $(B \times C)_{jk}$ = interactions between carbohydrase and lipase addition; these above components were tested using $e_m(a \times b \times c)$ as error term, and $e_m(a \times b \times c)$ = effect of repeated measurements (different intestinal segments) within the same bird m; D_l = effect of intestinal segment (jejunum, ileum); $(A \times D)_{il}$ = interactions between fat type and

intestinal segment; $(B \times D)_{jl}$ = interactions between carbohydrase addition and intestinal segment; $(C \times D)_{kl}$ = interactions between lipase addition and intestinal segment; e_{ijklm} = error term.

All experimental data were subjected to the GLM Procedure of SAS (SAS Institute, 1986) as a complete randomized design. All statements of significance are based on a probability of less than 0.05.

5.4. Results

5.4.1. Growth Performance

The results of growth performance of the broilers during the 2-wk experimental period are presented in Table 18. Birds fed diets containing beef tallow consumed 3.4% more ($P < 0.05$) feed to achieve a similar ($P > 0.05$) BW gain to chicks fed canola oil-containing diets. As a result, a poorer ($P < 0.001$) feed-to-gain ratio was noted for the diets containing tallow. Carbohydrase addition improved ($P < 0.001$) BW gain and feed-to-gain ratio by 5.4% and 3.4%, respectively. There was no effect of lipase addition on chicken performance. No interactions were observed between fat type and enzyme supplementations.

5.4.2. Nutrient Utilization

The results of the apparent digestibility of fat, starch, nitrogen, and NSP in different segments of the small intestine are summarized in Table 19. An effect ($P < 0.001$) of fat type was only observed for fat digestibility, which was 5.7% lower for tallow-containing diets than for canola oil-containing diets. Carbohydrase enzyme addition improved ($P < 0.001$) the digestibility of fat, starch, nitrogen, and NSP,

TABLE 18. Effect of dietary fat type, carbohydrase, and lipase addition on growth performance of broilers (5-18 d) fed wheat-based diets

Effect	Feed intake (g/bird)	BW gain (g/bird)	Feed:gain (g feed/g gain)
Fat type			
Tallow	698	476	1.47
Canola oil	675	482	1.40
Carbohydrase addition			
–	682	466	1.46
+	692	491	1.41
Lipase addition			
–	678	475	1.44
+	695	482	1.43
Pooled SEM	7.6	3.9	0.006
Source of Variation		Probability[1]	
Fat type	0.037	0.248	<0.001
Carbohydrases	0.362	<0.001	<0.001
Lipase	0.113	0.184	0.122
Fat type × carbohydrases	0.937	0.882	0.799
Fat type × lipase	0.387	0.633	0.219
Lipase × carbohydrases	0.542	0.563	0.624
Fat type × lipase × carbohydrases	0.894	0.985	0.729

[1]An effect with a probability of less than 0.05 is considered significant.

TABLE 19. Effect of dietary fat type, carbohydrase, and lipase addition on apparent digestibility (%) of fat, starch, nitrogen, and nonstarch polysaccharides (NSP) in the jejunum and the ileum of broilers fed wheat-based diets

Effect	Fat	Starch	Nitrogen	NSP
Fat type				
Tallow	68.8	88.7	55.0	10.1
Canola oil	72.7	89.7	56.8	12.2
Carbohydrase addition				
–	68.0	87.3	53.5	6.4
+	73.5	91.1	58.4	15.9
Lipase addition				
–	70.8	89.4	56.0	11.8
+	70.8	89.0	55.9	10.5
Pooled SEM	0.61	0.44	0.83	0.88
Fat type × carbohydrases				
Tallow				
–	64.8			
+	72.9			
Canola oil				
–	71.3			
+	74.1			
Pooled SEM	0.86			
Carbohydrases × intestinal segment				
Jejunum				
–	58.5	81.5	34.0	-0.7
+	66.3	87.1	41.2	11.8
Ileum				
–	77.6	93.0	72.9	13.6
+	80.7	95.1	75.5	20.0
Pooled SEM	0.66	0.60	0.63	0.97
Source of variation	Probability[1]			
Fat type	<0.001	0.129	0.134	0.110
Carbohydrases	<0.001	<0.001	<0.001	<0.001
Lipase	0.963	0.491	0.926	0.319
Fat type × carbohydrases	0.004	0.498	0.655	0.110
Fat type × lipase	0.855	0.610	0.659	0.474
Lipase × carbohydrases	0.861	0.795	0.502	0.826
Intestinal segment	<0.001	<0.001	<0.001	<0.001
Fat type × intestinal segment	0.727	0.959	0.095	0.094
Carbohydrases × intestinal segment	0.002	0.005	0.001	0.004
Lipase × intestinal segment	0.959	0.341	0.934	0.397

[1]An effect with a probability of less than 0.05 is considered significant.

irrespective of fat type. However, lipase addition had no effect ($P > 0.05$) on these parameters. An interaction ($P < 0.01$) between fat type and carbohydrase addition was only noted for fat digestibility, with a greater improvement for the tallow-containing diets. Interactions ($P < 0.01$) between carbohydrase enzyme and intestinal segment were observed for fat, starch, nitrogen and NSP digestibilities; the improvements due to carbohydrase enzyme addition were greater in the jejunum than in the ileum.

The water-soluble and water-insoluble NSP concentrations in different intestinal segments are shown in Table 20. Fat type and lipase addition had no effect ($P > 0.05$) on the concentrations of both NSP fractions, but carbohydrase addition increased ($P < 0.001$) soluble NSP and decreased ($P < 0.001$) insoluble NSP concentration. Interactions ($P < 0.05$) between carbohydrase addition and intestinal segment were also noted for soluble and insoluble NSP concentration, with a greater increase in soluble NSP and a greater decrease in insoluble NSP concentration in the jejunum than in the ileum.

Total tract digestibility of fat and NSP, jejunal digesta viscosity, and AME_n content of diets are shown in Table 21. Tallow-containing diets had a lower ($P < 0.001$) total tract digestibility of fat, which was paralleled by a lower ($P < 0.001$) AME_n content when compared to canola oil-containing diets. As opposed to the lipase addition, which showed no effect, the carbohydrase addition improved fat ($P < 0.001$) and NSP ($P < 0.05$) digestibilities, increased AME_n content ($P < 0.001$), and reduced ($P < 0.001$) jejunal viscosity. An interaction between fat type and carbohydrase addition was again only noted for fat digestibility ($P < 0.001$), the enzyme effect being more pronounced for tallow-containing diets.

TABLE 20. Effect of dietary fat type, carbohydrase, and lipase addition on water-soluble nonstarch polysaccharides (SNSP) and water-insoluble nonstarch polysaccharides (INSP) concentration (mg/g) in the jejunum and the ileum of broilers fed wheat-based diets

Effect	SNSP	INSP
Fat type		
Tallow	22.7	182
Canola oil	22.2	178
Carbohydrase addition		
−	24.6	197
+	20.4	164
Lipase addition		
−	22.4	180
+	22.6	180
Pooled SEM	0.36	5.3
Carbohydrases × intestinal segment		
Jejunum		
−	14.7	154
+	20.2	112
Ileum		
−	26.0	240
+	28.9	216
Pooled SEM	0.50	3.6
Source of variation	Probability[1]	
Fat type	0.327	0.608
Carbohydrases	<0.001	<0.001
Lipase	0.724	0.983
Fat type × carbohydrases	0.168	0.592
Fat type × lipase	0.283	0.704
Lipase × carbohydrases	0.390	0.770
Intestinal segment	<0.001	<0.001
Fat type × intestinal segment	0.901	0.197
Carbohydrases × intestinal segment	0.027	0.018
Lipase × intestinal segment	0.811	0.702

[1]An effect with a probability of less than 0.05 is considered significant.

TABLE 21. Effect of dietary fat type, carbohydrase, and lipase addition on total tract digestibility of fat and nonstarch polysaccharides (NSP), AME_n content, and jejunal digesta viscosity of broilers fed wheat-based diets

Effect	Fat (%)	NSP (%)	AME_n (kcal/kg)	Viscosity (mPa s)
Fat type				
Tallow	84.1	20.7	3048	3.9
Canola oil	88.0	22.1	3115	3.4
Pooled SEM	0.23	0.94	11.0	0.20
Carbohydrase addition				
–	84.3	19.5	3032	5.0
+	87.8	23.3	3132	2.4
Pooled SEM	0.23	0.94	11.0	0.20
Lipase addition				
–	86.2	21.4	3085	3.7
+	85.9	21.3	3078	3.6
Pooled SEM	0.23	0.94	11.0	0.20
Fat type × carbohydrases				
Tallow				
–	81.5			
+	86.7			
Canola oil				
–	87.1			
+	88.9			
Pooled SEM	0.33			
Source of Variation	Probability[1]			
Fat type	<0.001	0.300	<0.001	0.098
Carbohydrases	<0.001	0.012	<0.001	<0.001
Lipase	0.304	0.952	0.650	0.781
Fat type × carbohydrases	<0.001	0.841	0.080	0.964
Fat type × lipase	0.465	0.812	0.860	0.957
Lipase × carbohydrases	0.464	0.864	0.775	0.950
Fat type× lipase × carbohydrases	0.175	0.356	0.787	0.332

[1]An effect with a probability of less than 0.05 is considered significant.

5.5. Discussion

The present study clearly demonstrated that performance of birds fed wheat-based diets was affected by fat type and that chicks fed tallow-containing diets had a poorer feed-to-gain ratio. Poorer feed-to-gain ratios were observed by Brenes et al. (1993b), Langhout et al. (1997) and Preston et al. (2001) when similar wheat-based diets were supplemented with beef tallow rather than vegetable oils. Nutrient digestibility results indicated that the difference observed in feed-to-gain ratio between beef tallow- and canola oil-containing diets was only due to the difference in fat digestibility, because digestibilities of starch, nitrogen, and NSP were not affected by fat type. It is well known that beef tallow is characterized by low digestibility, particularly in young birds (Ketels and DeGroote, 1989). Ward and Marquardt (1983) attributed such poor digestibility of tallow to the degree of saturation of its fatty acids. Beef tallow contains mainly palmitic, stearic acids (LCSFA), and unsaturated oleic acid (Danicke, 2001). Danicke (2001) suggested that the LCSFA in beef tallow are nonpolar and thus rely on an adequate presence of bile salts for efficient emulsification and micelle formation, which are essential for fat digestion and absorption. Conversely, crude canola oil is primarily composed of long-chain unsaturated oleic, linoleic, and linolenic acids (NRC, 1994), which can be easily absorbed even in the absence of bile salts (Garret and Young, 1975). Despite the need for bile salts to digest tallow, birds younger than 3 wk have been observed to produce inadequate secretions of bile acids, particularly when tallow is provided as dietary fat (Krogdahl, 1985). Support for this was given by Polin et al. (1980) and Fengler et al. (1988), who showed that feeding exogenous bile salts to chicks increased their ability to digest tallow. Hence, in the present study, the insufficiency of

bile salts may account for the observed lower fat digestibility in the chicks fed the tallow-containing diet.

The specific arrangement of the saturated and unsaturated fatty acids on the glycerol moiety of a triglyceride molecule may also contribute to the observed differences between fat types. Pancreatic lipase shows specificity for the fatty acids esterified to glycerol in the 1- and 3- positions and leaves the 2-monoglycerides intact and absorbed in this form (Leeson and Summers, 2001). The 2-position LCSFA in the form of monoglycerides have greater solubility for micelle formation than the same fatty acids released from the 1- or 3- position, which are more nonpolar and insoluble and thus less digestible. Sibbald and Kramer (1977) reported that in beef tallow, 73 to 81% of palmitic and stearic acids are bound at the 1- and 3-positions, whereas the long-chain unsaturated fatty acids (mainly oleic acid) are esterified at the 2-position. This may also be an important factor contributing to the poorer fat digestibility of tallow-containing diets observed in the present study. Evidently, the lower AME_n value of tallow-containing diets, compared with canola oil-containing diets, is a consequence of a lower fat digestibility.

The lack of responses in chicken performance and fat digestibilities to the lipase addition suggests that the insufficiency of pancreatic lipase production may not contribute to the lower fat digestibility of tallow-containing diets in the current study. Examination of the published data indicates that although the daily net secretion of lipase into the duodenum increases significantly as the bird ages (Noy and Sklan, 1995), the secretion of lipase when calculated per gram of feed intake is less dramatic (Uni et al., 1996). This indicates that the lipase secretion of young birds may not be as inadequate as

expected when their feed intake is considered (Sklan, 2001). Although some increase in diet AME content and fat digestibility with lipase supplementation has been reported, lipase addition caused a significant reduction in feed intake and consequently lowered BW gain (Al-Marzooqi and Leeson, 1999). This was not the case in the current study, as performance parameters were similar to the control diets and no reduction in feed intake was observed. It would appear that such an "anorexic effect" observed in the earlier study may have been a consequence of the high inclusion rates of lipase (i.e., Pancreatic preparation). This was not the case in the current study because enzyme addition, when calculated per gram of feed intake, accounted for approximately 30% of the endogenous lipase secretion to the duodenum of the young chicken.

It has been shown that the antinutritional effect of wheat arabinoxylan amplifies the digestibility differences between fat types (Choct and Annison, 1992b; Choct et al., 1996). The relatively small difference in fat digestibility between tallow- and canola oil-containing diets (10% at intestinal level and 7% at fecal level, Table 19 and 21, respectively) in the absence of carbohydrase addition may be due to the relatively lower fat inclusion rate and a less viscous dietary background [jejunal viscosity of 5.2 and 4.7 mPa s (data not shown), respectively] when compared with other studies where fat digestibility difference of 29% (Danicke et al., 1997; Langhout et al., 1997) and 36% (Preston et al., 2001) were noted. Consequently, the growth rate of birds fed tallow-containing diets was depressed in these studies when compared with vegetable oil-containing diets. In the current study, however, BW gain was not affected by fat type, suggesting a less dramatic dietary situation. Under such condition, the birds fed tallow-

containing diets achieved a similar growth rate to those fed canola oil-containing diets by increasing feed intake to compensate for the compromised fat digestibility.

Significant interactions between fat type and carbohydrase addition for fat digestibility were detected in the current study at the intestinal and total tract levels. For example, at the intestinal level, carbohydrase supplementation improved fat digestibility by 12.5% (from 64.8 to 72.9%) in tallow-containing diets, but an improvement of only 3.8% (from 71.3 to 74.1%) was noted for canola oil-containing diets, suggesting that the degree of enzyme effect on fat digestibility is directly related to the type of dietary fat. Although the exact mechanism of action of dietary carbohydrases is not clear, their beneficial effects have been associated with the reduction of digesta viscosity caused by water-soluble and viscous NSP (Bedford and Classen, 1992). Water-soluble arabinoxylan of wheat has been shown to increase intestinal viscosity and to exert antinutritive effects (Choct and Annison, 1992b). Smulikowska (1998) suggested that an increased intestinal viscosity might lead to reduced gut motility, which in turn may decrease the rate of diffusion and the convective transportation of emulsion droplets, fatty acids, mixed micelles, bile salts and lipase within the small intestine. Such a situation would be particularly detrimental for the digestion of LCSFA, as they rely more on vigorous digesta mixing for optimal emulsification. In addition, Krogdahl (1985) suggested that both palmitic and stearic fatty acids are nonpolar and cannot spontaneously form mixed micelles, but can be solubilized into micelles formed from unsaturated fatty acids and conjugated bile salts. Therefore, such dependence of digestion and absorption of LCSFA upon the presence of bile salts, unsaturated fatty acids, and formed micelles would explain why tallow digestion is more sensitive to small increases in intestinal viscosity, as

observed in the present study. Pasquier et al. (1996) demonstrated that triglyceride hydrolysis and the amount of emulsified lipids were reduced in vitro as the viscosity of solution containing different concentrations of soluble fibre increased from 0 to 20 mPa s. The sensitivity of tallow digestion to viscosity would explain the more pronounced carbohydrase enzyme effect on improving fat digestibility of tallow-containing diets by reducing digesta viscosity from 5.0 to 2.4 mPa s. Notably, the significant interaction between fat type and carbohydrase addition occurred at an intestinal viscosity level of below 10 mPa s, a level most likely encountered with broilers fed practical Canadian wheat-based diets (Slominski et al., 2000). These results seem to indicate that the negative effect of viscous NSP on animal fat digestion is substantial in a practical wheat-based diet, and even relatively small reductions in viscosity due to enzyme action could greatly improve animal fat digestion and absorption.

Choct et al. (1996) also attributed a poorer digestibility of saturated, compared with unsaturated fatty acids, to increased intestinal fermentation. These authors suggested that, due to the hindrance of nutrient digestion by the viscous intestinal environment, more unabsorbed material reaches the ileum, which promotes the proliferation of detrimental microflora. Intestinal bacteria can deconjugate bile acids (Christle et al., 1997; Smits et al., 1998) making the bile acids inactive in fat emulsification and micelle formation (Smulikowska, 1998), and leading to a further depression in lipid digestion. This might be the case in the present study, where antibiotic-free diets were used. Studies by Kritchevsky and Story (1974) and Kritchevsky (1978) have demonstrated that fibrous foods bind bile acids and thereby increase their excretion. The viscous wheat arabinoxylan might exert a similar effect on bile acids and increase bile acid excretions

(Langhout et al., 1997). The problems described above might further exacerbate the deficiency of bile salts in the young broilers, but to what extent they contributed to the more depressed tallow digestion cannot be ascertained from the current data. The fact that the fat type × carbohydrase interactions were not detected for other nutrient digestibilities, AME_n value or growth performance confirms previous findings that digestion of fat is more affected by the negative effects of viscosity than that of other nutrients (Campbell et al., 1983; Choct and Annison, 1992b).

The present study clearly demonstrated that carbohydrase addition had an overall significant effect on nutrient digestibilities, AME_n, and growth performance of birds fed wheat-based diets. Similar results were observed in earlier studies when enzyme preparations were supplemented to broilers fed wheat-based diets (Annison, 1992; Friesen et al., 1992; Marquardt et al., 1994; Steenfeldt et al., 1998a, b). These authors attributed the beneficial effects of enzyme supplementation partially to depolymerization of soluble NSP into smaller polymers, and the resultant reduced digesta viscosity. This was evident in the current study, with the improvements in nutrient use and growth performance accompanied by a significantly increased NSP digestibility and a decreased digesta viscosity, both of which would be the consequence of breakdown of soluble NSP by the action of enzymes. Such effects of carbohydrase enzymes on reducing viscosity have been suggested to alleviate the constraints on diffusion of substrates, enzymes and products (Fengler and Marquardt, 1988), leading to enhanced nutrient digestion.

Enzymatic disruption of cell wall structure may accelerate digestion by allowing the rapid access of endogenous enzymes to cell wall encapsulated nutrients, and this might also contribute to the observed improvements in the present study as indicated by

the intestinal NSP concentration. A significant increase in soluble NSP and a decrease in insoluble NSP concentration were noted in the small intestine, indicating a partial breakdown of the plant cell wall structure. In earlier studies from this laboratory (Manuscript 1), it was demonstrated that the carbohydrase cocktail used in the current study was capable of depolymerizing cell wall NSP in wheat, soybean meal, canola meal and peas in vitro. The carbohydrase cocktail was effective in improving nutrient digestibility and growth performance of broilers fed a diet similar to that used in the present study. Because soybean meal, canola meal, and peas accounted for approximately 30% of the basal diet, enhanced digestion of these protein supplements, other than wheat, may also contribute partially to the overall enzyme effects. Therefore, it might be concluded that the positive effect of carbohydrase supplementation was a combination of released intracellular encapsulated nutrients through disruption of cell wall polysaccharides and of reduced digesta viscosity.

It is noteworthy that a significant interaction between carbohydrase addition and intestinal segment was observed for the digestibility of fat, starch, nitrogen, and NSP; the improvements in nutrient digestibilities were more pronounced in the jejunum than in the ileum when diets with enzyme were compared with diets without enzyme supplementation. This would indicate a shift of nutrient digestion and absorption toward the upper region of the small intestine in enzyme-supplemented broilers and would thus limit microbial growth in the hindgut due to a substrate limitation. This is of practical importance, as such enzyme effects would be more pronounced for poorly digestible rather than readily digestible ingredients. Therefore, one of the most important benefits of enzyme use would be a reduced variation in nutrient digestion.

In conclusion, the findings of the current study suggest that the negative effects of soluble NSP on animal fat digestion is substantial even in a practical wheat-based broiler diet known to result in low intestinal viscosity. This finding was supported by the detection of the interaction between fat type and carbohydrase addition for fat digestibility at the intestinal and total tract level. The importance of such interaction would be even more apparent with increasing dietary wheat and animal fat inclusion rates during the early growing period of broilers. This leads to a recommendation of supplementation with an appropriate carbohydrase preparation when an animal fat is used in diets based on viscous cereals. The multicarbohydrase cocktail may have exerted its beneficial effects on nutrient utilization and thus growth performance by decreasing intestinal viscosity and by eliminating the nutrient encapsulating effect of cell wall structural polysaccharides.

5.6. Acknowledgements

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6. MANUSCRIPT 4

The Use of Enzyme Technology to Improve Energy Utilization from Full-fat Oilseeds. Part I: Canola Seeds

6.1. Abstract

The effect of carbohydrase enzyme supplementation on energy utilization from full-fat canola seed was investigated in a TME_n assay with adult roosters and in a nutrient digestibility and growth performance study with broiler chickens. In the TME_n assay, enzyme preparations C (cellulase, 340 U/g), XG (xylanase, 63,600 U/g; glucanase, 48,300 U/g), P (pectinase, 10,000 U/g), and MC (mannanase, 10,900 U/g; cellulase 600 U/g) alone and in combination (C+P, C+XG, C+MC, P+XG, P+MC, XG+MC, C+P+XG, C+P+MC, and C+P+XG+MC) were evaluated at an inclusion level of 0.1%. On average, hammer-milled canola seed with a TME_n content of 3,642 kcal/kg showed an increase ($P < 0.05$) to 4,783 kcal/kg following supplementation with the most effective enzyme blends (C+P+XG, C+P+MC, and C+P+XG+MC). A similar pattern of increase ($P < 0.05$) in fat (80.4 vs 63.5%) and nonstarch polysaccharide (NSP) (20.4 vs 4.4%) digestibilities was observed. Enzyme combination C+P+XG was further evaluated in a 2-wk (5-18 d) trial with broiler chickens fed iso-nitrogenous and iso-energetic corn-soybean meal based diets containing canola seed (15%), the corresponding canola meal (8.85%) plus canola oil (6.15%) mixture, or canola seed (15%) supplemented with 3 different levels (0.002, 0.01, 0.05%) of the enzyme. Poorer ($P < 0.05$) feed-to-gain ratio (1.412 vs 1.344), lower ($P < 0.05$) total tract DM (65.9 vs 70.7%) and fat (69.6 vs 88.0%) digestibilities, AME_n content (2,963 vs 3,200 kcal/kg), and ileal fat (65.6 vs 85.6%) and protein (75.6 vs 81.2%) digestibilities were observed for the canola seed diet when compared with the canola meal plus canola oil diet. Enzyme supplementation of the canola seed diet resulted in an improvement ($P < 0.05$) in feed-to-gain ratio, total tract DM, fat and NSP digestibilities, AME_n content, and ileal fat digestibility. Although the enzyme effect on ileal and total tract fat digestibilities was significant at both high and

medium inclusion levels, other parameters showed the significant difference only when the highest inclusion rate of enzyme was used. These data support the need for carbohydrase enzyme supplements in poultry diets containing full-fat canola seed.

6.2. Introduction

There is growing interest within the feed industry to use full-fat oilseeds in poultry diets. The two major oilseeds grown in Canada are canola seed and flaxseed. Full-fat canola seed contains approximately 40% oil and 22% protein and is therefore a valuable source of energy and protein for poultry diets (Leeson et al., 1978; Shen et al., 1983; Salmon et al., 1988). In addition to providing a considerable amount of energy, the oil of canola seed is an excellent source of α-linolenic acid (18:3ω3, 8-12%; Ajuyah et al., 1991), which has been shown to be important for human health (Sim et al., 1991; Ajuyah et al., 1991). Canola seed and off-grades of canola seed could also be viewed as an economically viable alternative to crude canola oil in poultry diets. In addition, the seeds are often available locally and in the intact form are more resistant to oxidation and can be more easily handled in on-farm feed mills than other ordinary fat sources (Leeson et al., 1978; Sim et al., 1991).

The feeding value of full-fat canola seed has been evaluated in several studies and it has been demonstrated that up to 10% of raw canola seed could be used in diets for broiler chickens, layers and turkeys (Leeson et al., 1978; Salmon et al., 1988; Ajuyah et al., 1991). However, the metabolizable energy content of ground canola seed has been demonstrated to be lower than that of the corresponding canola meal-canola oil mixture (Lee et al., 1991; 1995). Summers et al. (1982) reported that feeding 17.5% of full-fat canola seed to broiler chickens resulted in depressed fat utilization and weight gain. Such

reduced energy utilization from canola seed has been suggested to be due to a lower oil availability resulting from the oil encapsulating effect of the cell wall polysaccharides (Lee et al., 1991). Heat and mechanical treatments (e.g. flaking, steam pelleting, extrusion) of canola seed have proven beneficial in improving the feeding value of canola seed (Shen et al., 1983; Salmon et al., 1988).

Grinding is used to disrupt the cell wall structure of feedstuffs to increase the exposure of nutrients to the animal's digestive enzymes. In an earlier study from our laboratory (Jiang, 1999), an increase in available energy content was observed when the average particle size of a coarsely ground canola-quality mustard seed was reduced from 2.0 to 0.6 mm. A highly significant effect of particle size on apparent digestibility of nutrients by broiler chickens and laying hens fed full-fat canola seed has been reported (Danicke et al., 1998). Due to the high oil content and the small seed size, the grinding of canola seeds is a difficult procedure as the hammer mill will gum-up and when the seeds are premixed with cereal grains to overcome this problem, the grinding may not be sufficient for disruption of the cell wall structure. Consequently, the nutrient encapsulating effect of the cell walls may not be overcome by poultry as they lack enzymes to digest polysaccharides of the cell walls.

Supplementation of carbohydrase enzymes has been used to target cell wall/nonstarch polysaccharides (NSP) of feedstuffs in poultry diets. An in vitro study in Manuscript 1 demonstrated that a significant depolymerization of the cell wall polysaccharides of canola meal was achieved when different cell wall-degrading carbohydrase enzymes were used in concert. This was further substantiated in a TME_n assay in which the sample of a coarsely ground canola seed processed by a local egg producer showed TME_n content of 4,176 kcal/kg which increased significantly to 4,744

kcal/kg following enzyme supplementation (Manuscript 1). Therefore, the objective of the current research was to further investigate the effectiveness of carbohydrase enzymes in improving energy utilization from full-fat canola seed and thus enhance its feeding value for poultry.

6.3. Materials and Methods

6.3.1. TME_n Assay

The sample of raw canola seed used in the study was obtained from a local feed manufacturer in Manitoba, Canada and was hammer-milled to pass through a 2 mm sieve using a Wiley mill standard model No.3 grinder[1]. Although the diameter of canola seed is usually less than 2 mm, no intact seeds were present in the samples. Enzyme preparations C (cellulase, 340 U/g), XG (xylanase, 63,600 U/g; glucanase, 48,300 U/g), P (pectinase, 10,000 U/g), MC (mannanase, 10,900 U/g; cellulase 600 U/g) alone and in combination (C+P, C+XG, C+MC, P+XG, P+MC, XG+MC, C+P+XG, C+P+MC, and C+P+XG+MC) were evaluated in the assay. The activity units of enzymes were defined in Manuscript 1. Enzymes were included in the canola seed samples at a level of 0.1%. In the case of enzyme combinations, an equal portion of each enzyme (i.e., 1:1 w/w) was used. The enzyme products were provided by Canadian Bio-System Inc., Calgary, Alberta, Canada.

Nitrogen corrected true metabolizable energy content and fat and NSP digestibilities of canola seed without or with enzyme supplementation were determined using the assay procedure described by Sibbald (1986) with some modifications (Zhang et al., 1994). Briefly, each sample was precision-fed (25 g per bird) to 7 individually

[1] Authur H. Thomas Company, Philadelphia, USA.

caged, mature Single Comb White Leghorn cockerels 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 30 birds fed 50 mL of a 50% glucose solution (25g of dry glucose) were used to determine the endogenous excretion of energy and nitrogen. For the purpose of statistical analysis, 3 most effective enzyme combinations (C+P+XG, C+P+MC, and C+P+XG+MC) were evaluated using 3 groups of 7 birds per treatment.

6.3.2. Performance and Nutrient Utilization Study

The effect of enzyme combination C+P+XG was further evaluated in a growth performance and nutrient utilization experiment with broiler chickens. The dietary treatments included a canola meal (8.85%) plus canola oil (6.15%) diet, a canola seed (15%) diet, and the canola seed diet supplemented with enzyme C+P+XG at 3 different levels: 0.002, 0.01, and 0.05%. Based on the measured fat content (41.0%) of the seed, the diet with canola meal plus canola oil was formulated to contain the same meal to fat ratio as that present in the seed. The seeds were ground to pass through a 2 mm sieve as described in the TME_n assay section. All the experimental diets were corn-soybean meal based and were formulated to be iso-nitrogenous and iso-energetic. The composition of the basal diet is shown in Table 22. Chromic oxide (3.0 g/kg) was mixed with the diets and 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

TABLE 22. Composition and calculated analysis of basal diets (g/kg)

	Canola meal + canola oil diet	Canola seed diet
Ingredient		
Corn (8.5% CP)	485.0	485.0
Soybean meal (43% CP)	318.0	318.0
Canola meal (36% CP)	88.5	
Canola seed (20% CP)		150.0
Canola oil	61.5	
Limestone[1]	12.5	12.5
Dicalcium phosphate[2]	15.5	15.5
DL-methionine	1.0	1.0
L-lysine-HCl		
Mineral premix[3]	5.0	5.0
Vitamin premix[4]	10.0	10.0
Chromic oxide	3.0	3.0
Total	1,000.0	1,000.0
Calculated analysis		
Crude protein (%)[5]	21.0	21.0
AME (kcal/kg)	3,055.0	3,056.0
Lysine (%)	1.12	1.14
Methionine (%)	0.49	0.49
Methionine + cystine (%)	0.87	0.84
Calcium (%)	0.96	0.95
Available Phosphorous (%)	0.43	0.43

[1]Contained 38% calcium.

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

[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.

brooders[2] for a 4-d pre-experimental period and fed commercial chick starter crumbles (21% CP). On d 5, birds were fasted for 4 h, individually weighed and randomly distributed among the 5 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 the 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 the 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 day 19, 24 birds were randomly selected from each treatment group and killed by cervical dislocation. The contents of ileum (from Meckel's diverticulum to 1cm above the ileo-caecal junction) were collected and pooled for 4 birds to yield 6 replicate samples per treatment. 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 and the protocol for this study was approved by the animal care committee of the University of Manitoba.

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[2] James Mfg. Co., Mount Joy, PA.

6.3.3. 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[3] 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[4]. 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 hrs.

Nonstarch polysaccharide levels were determined by gas-liquid chromatography (component neutral sugars) and by colorimetry (uronic acids). The procedure for neutral sugars was performed as described by Englyst and Cummings (1988) with some modifications (Slominski and Campbell, 1990). Uronic acids were determined using the procedure described by Scott (1979).

6.3.4. Calculations and Statistical Analysis

In the performance and nutrient utilization study, the following equations were used for the 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):

Total tract NSP digestibility (%) = {1 – [(Cr_2O_3 % diet / Cr_2O_3 % excreta) × (NSP % excreta /NSP % diet)]} ×100,

Ileal fat digestibility (%) = {1 – [(Cr_2O_3 % diet / Cr_2O_3 % digesta) × (Fat % digesta /Fat % diet)]} ×100,

[3] LECO Corp., St. Joseph, MI.
[4] Parr Instrument Co., Moline, IL

AME_n (kcal/kg of diet) = GE kcal/kg diet – [GE kcal/kg excreta × (Cr_2O_3 % diet / Cr_2O_3 % excreta)] – 8.22 × {N % diet – [N % excreta × (Cr_2O_3 % diet / Cr_2O_3 % excreta)]},

Where GE is gross energy, N is nitrogen, Cr_2O_3 is chromic oxide, and 8.22 is the energy equivalent of uric acid nitrogen, that is, 8.22 kcal/kg of uric acid nitrogen.

Both the TME_n assay and the nutrient utilization and performance study were set up as completely randomized designs and data were subjected to ANOVA using the GLM procedure of SAS (SAS Institute, 1986) program. Means were separated by using Duncan's multiple range test (Snedecor and Cochran, 1980). Regression analysis on all the seed samples originally evaluated in the TME_n assay was performed using GLM (SAS Institute, 1986). All the statements of significance are based on a probability of less than 0.05.

6.4. Results and Discussion

The results of the TME_n assay demonstrated a substantial increase in energy availability following enzyme supplementation. The ground seed using the hammer mill technology showed, on average, an increase in TME_n content from 3,642 to 4,700 kcal/kg with a range from 4,536 to 5064 kcal/kg for all carbohydrase enzymes evaluated (individually or in combinations). As demonstrated in Figure 1, the improvement in energy availability with enzyme supplementation was directly related to improved fat digestibility ($r^2 = 0.94$; $P < 0.0001$) and as shown in Figure 2, was highly correlated with the improved NSP digestibility ($r^2 = 0.84$; $P < 0.0001$). This finding clearly demonstrates the importance of nutrient encapsulating effect of cell walls on energy utilization and

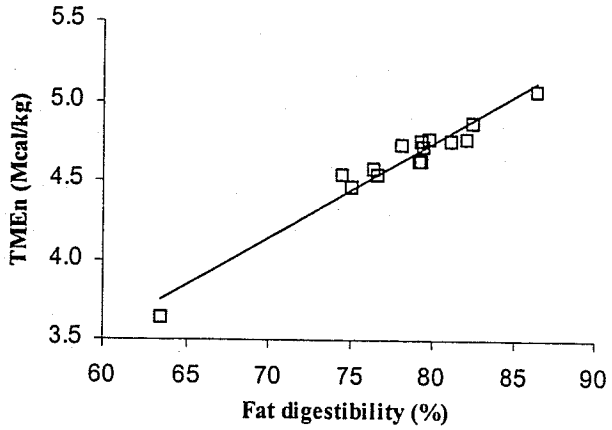

FIGURE 4. Relationship between TME_n content and fat digestibility in roosters fed full-fat canola seed supplemented with different combinations of carbohydrase enzymes. $r^2 = 0.94$; $P < 0.0001$. $Y = 0.059x$.

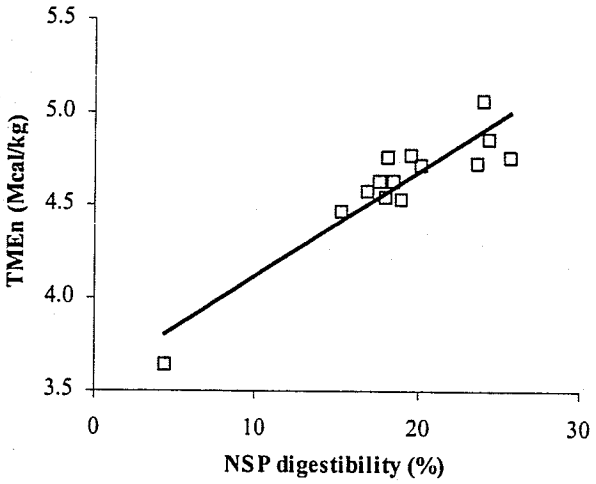

FIGURE 5. Relationship between TME_n content and nonstarch polysaccharide (NSP) digestibility in roosters fed full-fat canola seed supplemented with different combinations of carbohydrase enzymes. $r^2 = 0.84$; $P < 0.0001$. $Y = 3.56 + 0.056x$.

exemplifies the importance of carbohydrase enzyme use in the elimination of such an effect.

When using the most effective enzyme blends (C+P+XG, C+P+MC, and C+P+XG+MC), the TME_n content of canola seed averaged 4,783 kcal/kg with no significant differences between the enzyme supplements (Table 23). The increase in energy availability was accompanied by a similar pattern of increase ($P < 0.05$) in apparent fat and NSP digestibilities in roosters receiving the enzyme-supplemented canola seeds (Table 23).

The TME_n value obtained in the present study for the coarsely ground canola seed without enzyme supplementation (3,642 kcal/kg) was much lower than that of 4,637 kcal/kg determined in our earlier study for the finely ground canola seed (Manuscript 1). It was also lower than the values of 4,560 and 4,578 kcal/kg reported by Barbour and Sim (1991) and Lee et al. (1995), respectively. Such discrepancy may have resulted from a finer grind of the seed samples used in the energy availability assays, although the details of the grinding procedures used in the last two studies were not specified. It is evident from the current study that, although the conventional grinding process has positive effects on seed rupture and cell wall disruption, some portion of the oil may still be encapsulated by the cell wall structure and may require enzyme supplementation for optimum energy utilization of full-fat canola seed. Although the information on enzyme application in full-fat canola seed is scarce, the results of the present study are in agreement with the findings of some studies, which demonstrated that addition of multicarbohydrase enzyme cocktails to poultry diets were effective in improving NSP digestibility of canola meal (Slominski and Campbell, 1990; Simbaya et al., 1996; Kocher et al., 2000; Manuscript 2).

TABLE 23. Effect of different combinations of carbohydrase enzymes on digestibility of fat and nonstarch polysaccharides (NSP) and TME_n content of canola seed fed to adult roosters

Enzyme[1]	Fat (%)	NSP (%)	TME_n (kcal/kg)
None (control)	63.5[2b]	4.4[b]	3642[b]
C + P + XG	82.4[a]	24.4[a]	4869[a]
C + P + MC	79.4[a]	20.2[a]	4718[a]
C + P +XG + MC	79.3[a]	25.8[a]	4761[a]
SEM	1.52	2.56	40.9

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

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

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

The growth performance of broiler chickens fed diets containing either canola meal plus canola oil mixture or canola seed supplemented with different levels of enzyme C+P+XG is shown in Table 24. Although there was no difference in feed intake and body weight gain, the feed-to-gain ratio of broilers fed the canola seed diet was poorer ($P < 0.05$) than that of birds fed the corresponding canola meal plus canola oil diet. The improvement in feed efficiency was noted for the enzyme-supplemented canola seed diets, although the difference from that of the non-enzyme treatment was only significant ($P < 0.05$) for the highest level of the enzyme blend used.

The results of the apparent total tract digestibility of DM, fat and NSP, the AME_n content of experimental diets, and ileal fat and protein digestibilities are shown in Tables 25 and 26. A similar NSP digestibility was found for broiler chickens fed the canola seed diet and the canola meal plus canola oil diet. This is expected since, in general, birds lack endogenous enzymes to digest NSP. When compared with the canola meal plus canola oil diet, reduced ileal fat and protein and total tract DM and fat digestibilities and consequently a lower AME_n value was found for the canola seed-containing diet. These results clearly demonstrate the incomplete energy utilization from canola seed due to nutrient encapsulating effect of the cell wall polysaccharides. This may have resulted from insufficient disruption of the oil containing cells during the grinding process or in the gizzard of the bird with part of the oil escaping digestion in the small intestine. Similar results were reported by Lee et al. (1995) who demonstrated lower metabolizable energy values for canola seed when compared to the corresponding canola meal plus canola oil mixtures. It would also appear evident that the extensive rupture of the cotyledon fraction of canola seed during the commercial oil extraction process may result in more complete cell opening with more protein being available for digestion in the

TABLE 24. Growth performance of broiler chickens (d 5 to 18) fed diets containing canola meal plus canola oil mixture or canola seed supplemented with different levels of a multicarbohydrase enzyme

Treatment	Feed intake (g/bird)	Body weight gain (g/bird)	Feed-to-gain ratio
Canola meal + canola oil diet	675.1[1]	505.0	1.344[c]
Canola seed diet	702.3	497.0	1.412[a]
Canola seed diet + Low level enzyme[2] (0.002%)	684.9	492.8	1.388[ab]
Canola seed diet + Medium level enzyme (0.01%)	705.1	513.1	1.372[b]
Canola seed diet + High level enzyme (0.05%)	692.8	505.9	1.370[b]
SEM	11.2	9.0	0.007

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

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

[a-c] Means within a column with no common superscripts differ ($P < 0.05$).

TABLE 25. Apparent total tract dry matter (DM), fat and nonstarch polysaccharide (NSP) digestibilities and AME_n content of experimental diets fed to broiler chickens

Treatment	DM (%)	Fat (%)	NSP (%)	AME_n (kcal/kg diet)
Canola meal + canola oil diet	70.7[1a]	88.0[a]	11.6[bc]	3200[a]
Canola seed diet	65.9[b]	69.6[d]	10.5[bc]	2963[b]
Canola seed diet + Low level enzyme[2] (0.002%)	66.0[b]	71.7[d]	7.0[c]	2956[b]
Canola seed diet + Medium level enzyme (0.01%)	67.1[b]	74.7[c]	12.3[b]	3016[b]
Canola seed diet + High level enzyme (0.05%)	70.6[a]	77.5[b]	21.0[a]	3165[a]
SEM	0.73	0.80	1.50	25.0

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

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

[a-d] Means within a column with no common superscripts differ ($P < 0.05$).

TABLE 26. Apparent ileal digestibility of fat and protein in broilers fed different diets

Treatment	Fat (%)	Protein (%)
Canola meal + canola oil diet	85.6[1a]	81.2[a]
Canola seed diet	65.6[d]	75.6[b]
Canola seed diet + Low level enzyme[2] (0.002%)	66.6[d]	72.2[b]
Canola seed diet + Medium level enzyme (0.01%)	71.0[c]	72.2[b]
Canola seed diet + High level enzyme (0.05%)	75.5[b]	76.6[b]
SEM	1.22	1.50

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

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

[a-d]Means within a column with no common superscripts differ ($P < 0.05$).

resultant meal. This may explain, in part, higher ileal protein digestibility values observed in the present study for birds fed the canola meal plus canola oil diet.

Enzyme supplementation resulted in a significant increase ($P < 0.05$) in apparent total tract digestibility of DM, fat and NSP and AME_n content of the canola seed diet. As observed for the feed-to-gain ratio, the enzyme effects on DM and NSP digestibilities and AME_n were only significant for the diets with the highest enzyme inclusion level. Ileal and total tract fat digestibilities were improved ($P < 0.05$) for birds fed the canola seed diet at both high and medium enzyme inclusion levels. However, no effect of enzyme supplementation on ileal protein digestibility was observed. The positive effect of carbohydrase enzyme supplementation was likely due to enhanced energy utilization as a consequence of the reduced nutrient encapsulating effect of the cell walls similar to that observed in the TME_n assay. It is evident from this study that a relatively high level of enzyme supplement is needed to achieve a significant response in birds fed canola seed-containing diets. This may be due to the complex structure of the cell wall NSP of canola as well as their low water-solubility and thus low accessibility for the enzyme action (Manuscript 1). It is important to note, however, that with enzyme supplementation the improvement in feed-to-gain ratio (1.37 vs 1.41) and ileal (75.5 vs 65.6%) and total tract (77.5 vs 69.6%) fat digestibilities did not reach the values observed for the canola meal plus canola oil diet (i.e., 1.34, 85.6, and 88.0%, respectively) although no difference in AME_n content was observed between canola meal plus canola oil diet and the enzyme supplemented canola seed diet (Table 25). Further research is needed to screen more effective enzyme blends and fine tune the enzyme inclusion rates for optimum energy (oil) utilization by broiler chickens fed diets containing coarsely ground canola seed.

In conclusion, the present study demonstrates a significant oil encapsulating effect of cell wall NSP resulting in less than optimum energy utilization from full-fat canola seed. The multicarbohydrase preparation containing cellulase, pectinase, mannanase, xylanase and glucanase activities was found effective in partially alleviating such an effect with a resultant improvement in the feeding value of full-fat canola seed for broiler chickens. This data support the need for carbohydrase enzyme supplements in poultry diets containing coarsely ground full-fat canola seed.

6.5. Acknowledgements

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7. MANUSCRIPT 5

The Use of Enzyme Technology to Improve Energy Utilization from Full-fat Oilseeds. Part II: Flaxseeds

7.1. Abstract

An in vitro incubation study was carried out to determine if 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; glucanase, 48,300 U/g), P (pectinase, 10,000 U/g), and MC (mannanase, 10,900 U/g; 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% of 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 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 nonenzyme supplemented 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 (5-18 d) trial with broiler chickens fed a corn-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-to-gain ratio, 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 to enhance its feeding value for poultry.

7.2. Introduction

Full-fat flaxseed contains approximately 40% oil and 22% protein and is a valuable source of energy and protein for poultry diets. In recent years, it has become an attractive feed ingredient in Canadian poultry diets because of its high content of omega-3 unsaturated fatty acids (48-58% 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 et al., 1995) and have a positive effect on human health (Hargis and Van Elswyk, 1993; Ferrier et al., 1995; Mayo et al., 1995). However, reduced energy utilization and depressed growth and feed efficiency have been observed when incorporating 10-20% ground flaxseed to 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 body weight, 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 the bird fed flaxseed. The less than optimum energy utilization from full-fat flaxseed might be a result of limited oil availability. In the conventionally ground flaxseed, a substantial amount of oil may be encapsulated by the cell wall/nonstarch polysaccharides (NSP).

Lee et al. (1991), on the other hand, observed a further depression in performance and energy utilization when the level of flax meal in the broiler diets increased from 6.5% to 13%. This indicates that the energy utilization 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, and phytic acid (Madhusudhan et al., 1986). Mucilage is a mixture of branched chain water-soluble polysaccharides, present in the hulls of flaxseed (3-9% 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) utilization from full-fat canola seed (Manuscripts 1 and 4). However, the effectiveness of carbohydrase enzymes on energy utilization 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 utilization from full-fat flaxseed when fed to adult roosters (TME_n assay) and broiler chickens.

7.3. Materials and Methods

7.3.1. In Vitro Enzyme Evaluation

An in vitro incubation study was carried out to determine if various carbohydrase preparations contained appropriate activities to target NSP of flaxseed. The sample of raw flaxseed was finely ground, defatted with hexane for 6 hr, and air-dried for use in the assay. Enzyme preparations C (cellulase, 340 U/g), XG (xylanase, 63,600 U/g; glucanase, 48,300 U/g), P (pectinase, 10,000 U/g), MC (mannanase, 10,900 U/g; 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 using the defatted flaxseed meal as the substrate. The activity units of enzymes were defined in Manuscript 1. The enzyme preparations were provided by Canadian Bio-System Inc., Calgary, Alberta, Canada. The in vitro incubation procedure applied in this study was as described in Manuscript 1. Briefly, triplicate samples (0.1 g) of the flaxseed meal were incubated with 1% of single enzymes or combinations in a 0.1 M sodium acetate buffer (pH 5.2) at 45°C for 16 hrs. In the case of enzyme combinations, an equal portion of each enzyme (i.e., 1:1 w/w) 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.

7.3.2. 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 utilization of full-fat flaxseed was evaluated in a TME_n assay. The sample of raw flaxseed used in the study was hammer-milled to pass through a 2 mm

sieve using a Wiley mill standard model No.3 grinder[1]. Enzymes were included in the flaxseed samples at a level of 0.1% and an equal portion of each enzyme (i.e., 1:1 w/w) was used for each enzyme combination.

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 (25g of dry glucose) were used to determine the endogenous excretion of energy and nitrogen.

7.3.3. Performance and Nutrient Utilization Study

The effect of the enzyme combination C+P+XG was further evaluated in a growth performance and nutrient utilization experiment with broiler chickens. The dietary treatments included a corn/soybean meal-based flaxseed (15%) diet, and the flaxseed diet supplemented with enzyme C+P+XG at 3 different 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 27. Chromic oxide (3.0 g/kg) was included in the diets and used to calculate nutrient digestibilities and AME_n content.

[1] Authur H. Thomas Company, Philadelphia, USA.

TABLE 27. Composition and calculated analysis of basal diet

	g/kg
Ingredient	
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 Phosphorous (%)	0.43

[1]Contained 38% calcium.

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

[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.

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[2] for a 4-d pre-experimental period and fed commercial chick starter crumbles (21% CP). On d 5, birds were fasted for 4 h, individually weighed and randomly distributed among the 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 calculated to determine the 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 the 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 day 19, 24 birds 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 1cm above the ileo-caecal 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 minutes and viscosity of the supernatant was determined at 40°C using the Brookfield digital

[2] James Mfg. Co., Mount Joy, PA.

viscometer[3]. 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 and the protocol for this study was approved by the animal care committee of the University of Manitoba.

7.3.4. 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[4] 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[5]. 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 hrs. NSP were determined as described in the In vitro Enzyme Evaluation section.

7.3.5. 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):

[3] Model DV-II+LV, Brookfield Engineering Laboratories, Stoughton, MA.
[4] LECO Corp., St. Joseph, MI.
[5] Parr Instrument Co., Moline, IL

Total tract NSP digestibility (%) = $\{1 - [(Cr_2O_3\ _{\%\ diet} / Cr_2O_3\ _{\%\ excreta}) \times (NSP\ _{\%\ excreta} / NSP\ _{\%\ diet})]\} \times 100$,

Ileal fat digestibility (%) = $\{1 - [(Cr_2O_3\ _{\%\ diet} / Cr_2O_3\ _{\%\ digesta}) \times (Fat\ _{\%\ digesta} / Fat\ _{\%\ diet})]\} \times 100$,

AME_n (kcal/kg of diet) = $GE\ _{kcal/kg\ diet} - [GE\ _{kcal/kg\ excreta} \times (Cr_2O_3\ _{\%\ diet} / Cr_2O_3\ _{\%\ excreta})] - 8.22 \times \{N\ _{\%\ diet} - [N\ _{\%\ excreta} \times (Cr_2O_3\ _{\%\ diet} / Cr_2O_3\ _{\%\ excreta})]\}$,

Where GE is gross energy, N is nitrogen, Cr_2O_3 is chromic oxide, and 8.22 is the energy equivalent of uric acid nitrogen, that is, 8.22 kcal/kg of uric acid nitrogen.

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

7.4. 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 28. The total NSP content of the flaxseed meal averaged 271 g/kg. Based on the measured fat content (42.5%) of the seed and less than 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 followed by arabinose (10%), galactose (10%), and rhamnose (6%).

TABLE 28. Degradation of nonstarch polysaccharides (NSP) following incubation of defatted flaxseed meal with different preparations of carbohydrase enzymes (g/kg)

Enzyme	Component sugar						Total NSP[1]
	Rhamnose	Arabinose	Xylose	Galactose	Glucose	Uronic acids	
None (control)	15.1[2a]	27.0[a]	57.3[a]	27.0[a]	80.1[a]	62.7[a]	271[a]
Cellulase (C)	15.6[a]	21.9[e]	48.2[cd]	22.5[c]	57.8[e]	54.2[cde]	222[e]
Pectinase (P)	12.7[b]	22.9[bc]	50.4[bc]	23.2[cb]	72.4[b]	53.0[de]	236[b]
Xylanase/glucanase (XG)	15.3[a]	23.8[b]	52.9[b]	24.0[b]	72.2[b]	53.7[cde]	243[b]
Mannanase/cellulase (MC)	14.8[a]	19.0[ef]	45.7[d]	21.4[d]	55.9[cd]	59.8[bc]	218[e]
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

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

[2]Means of triplicate determination.

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

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; Manuscript 1), 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 flaxseed meal with enzyme preparations C, P, XG, and MC resulted in a significant ($P < 0.05$) 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 ($P < 0.05$) of all the 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 ($P < 0.05$) 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 29. The hammer milled full-fat flaxseed showed an increase ($P < 0.05$) 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, with no significant differences among the 3 enzyme supplements. The

increase in energy availability was accompanied by a similar pattern of increase ($P < 0.05$) 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 utilization from the oil-containing cells. Therefore, supplementation with cell wall-degrading carbohydrase enzymes may serve as an attractive means of facilitating fat accessibility for digestion and thus enhancing the overall energy utilization from flaxseed.

The TME_n value obtained in the present study for the ground flaxseed without enzyme supplementation (2,717 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 (Manuscript 4), such discrepancy in TME_n values between our study and the previous studies may 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 employ a proper grinding procedure to ensure the optimum energy utilization. Since a small amount of sample is usually needed to perform the TME assay, fine grinding using laboratory mills is often the method of choice when evaluating the high oil-containing feedstuffs. However, under the commercial conditions, higher diameter sieves (i.e., 4 mm) are often used for oilseed processing since during grinding the seed gum-up the hammer-mills and, even if premixed with cereal grains, the grinding may still not be sufficient for a complete

TABLE 29. Effect of different combinations of carbohydrase enzymes on digestibility of fat and nonstarch polysaccharides (NSP) and TME_n content of flaxseed fed to adult roosters

Enzyme[1]	Fat (%)	NSP (%)	TME_n (kcal/kg)
None (control)	59.4[2b]	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

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

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

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

rupture of the cells. It is of interest to note that, in the current study, the TME_n value (3,750 kcal/kg) of the flaxseed when supplemented with enzymes was similar to those reported in earlier studies. It would suggest that following the conventional hammer-mill grinding, some portion of the oil may still be encapsulated by the cell wall structure and enzyme supplementation would further facilitate the seed 'grinding' which, in turn, would result in optimum energy utilization 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 30. Although feed intake and BW gain were not affected, the feed-to-gain ratio of broilers fed the flaxseed diet was improved ($P < 0.05$) following supplementation with the enzyme blend at the highest rate (0.05%). Enzyme supplementation resulted in a significant increase ($P < 0.05$) in apparent total tract digestibility of DM, fat and NSP and AME_n content of the flaxseed-containing diet when the enzyme blend was added at the highest level (Table 31). As observed for the feed-to-gain ratio, the enzyme effect was only significant for the diet with the highest enzyme level. The results of ileal fat and protein digestibilities and jejunal digesta viscosity are presented in Table 32. Ileal fat digestibility was improved ($P < 0.05$) when the enzyme inclusion rate was at 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-to-gain ratio (1.484), low fat digestibility (56.4% at the ileal level) and AME_n content (2,701 kcal/kg) were observed in the present study for the control diet. A marked depression in fat digestibility, AME_n, and growth performance has

TABLE 30. Growth performance of broiler chickens (d 5 to 18) fed diets containing flaxseed supplemented with different levels of a multicarbohydrase enzyme

Treatment	Feed intake (g/bird)	Body weight gain (g/bird)	Feed-to-gain ratio
Flaxseed diet	675.2[1]	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

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

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

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

TABLE 31. Apparent total tract digestibility of dry matter (DM), fat and nonstarch polysaccharides (NSP) and AME_n content of experimental diets fed to broiler chickens

Treatment	DM (%)	Fat (%)	NSP (%)	AME_n (kcal/kg diet)
Flaxseed diet	62.0[1b]	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

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

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

[a-c] Means within a column with no common superscripts differ ($P < 0.05$).

TABLE 32. Apparent ileal digestibility of fat and protein and jejunal digesta viscosity in broilers fed different diets

Treatment	Fat (%)	Protein (%)	Digesta viscosity (mPa s)
Flaxseed diet	56.4[1b]	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

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

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

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

also been previously reported for broilers fed flaxseed-containing diets (Ajuyah et al., 1991; Lee et al., 1991; Alzueta et al., 2003). Such inhibition of fat digestion may 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. 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., 1993b), may depress digestion and absorption of nutrients through increasing digesta viscosity due to 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 digestibility due to high gut viscosity has been reported to be the highest among the nutrients evaluated (Campbell et al., 1983; Choct and Annison, 1992b; Manuscript 3).

As 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 utilization 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 canola seed-containing diet (Manuscript 4), a relatively high level of enzyme supplementation is needed to achieve a significant response in flaxseed-containing diets, possibly due to the structural complexity of the flax NSP. However, both broiler chicken performance data and nutrient digestibility values were lower than

those observed earlier for the canola seed-containing diets (Manuscript 4). 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 may have inhibited fat digestion and absorption. Therefore, more research is needed to screen for more effective enzyme blends to further reduce the viscosity of flax mucilage.

In conclusion, the present studies demonstrate that multicarbohydrase preparations could be effective in degrading the cell wall polysaccharides and in improving energy utilization from flaxseed. Hence, carbohydrase enzyme supplements may be used as an attractive means of enhancing the feeding value of full-fat flaxseed for poultry.

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8. GENERAL DISCUSSION

One of the objectives of the current research was to evaluate the effectiveness of selected carbohydrase combinations in practical Canadian broiler diets based on wheat, SBM, and local vegetable protein supplements such as CM and peas. Such diets were used in two studies (Manuscripts 1 and 3) and showed an intestinal viscosity of 3.3 and 5.0 mPa s for the 51 and 60% wheat inclusion rates, respectively. This confirms the relatively low digesta viscosity reported previously for broilers fed Canadian wheat-based diets (Leeson et al., 2000; Slominski et al., 2000; McCracken and Miller, 2002). In one of the studies (Manuscript 3), the viscosity was reduced from 5.0 to 2.4 mPa s and this could be attributed to the significant effect of enzyme supplementation on improving nutrient digestibilities and thus growth performance of broilers. In the other study (Manuscript 1), however, the results indicated that the beneficial responses from carbohydrase enzyme supplementation may partially result from the elimination of nutrient encapsulating effect of cell wall NSP. This encapsulation effect seems to be more apparent in low-viscosity wheat. It would appear that carefully screened enzyme combinations having affinity for both soluble and insoluble NSP will be more efficacious. This supports the theory that both the viscous nature of NSP and the insoluble cell wall matrix encapsulating nutrients are important factors influencing the nutritive value of wheat for poultry.

The enzyme combinations used in the two studies (Manuscripts 1 and 3) were also selected based on their efficacy towards the NSP of SBM, CM, and peas. Therefore, it is speculated that the nutrient utilization from SBM, CM and peas may also be

improved by enzyme supplementation and this may have contributed to the significant improvements in growth performance and nutrient digestibilities in birds fed the wheat-SBM-CM-peas-based diets (Manuscripts 1 and 3). However, the results of another study where the same enzyme combination was supplemented to corn-based SBM, CM, or pea diets (Manuscript 2) suggest that the improvements may have resulted from the enzyme effects on wheat and, possibly, SBM rather than on CM or peas. This study also suggests that the nutrient encapsulating effect of the cell walls may not be responsible for incomplete nutrient utilization from SBM, CM, or peas due to sufficient rupture of the cell wall structure of soybean and canola during the oil extraction process while in the case of peas, due to the resistant nature of pea starch. It was noted that in all cases although the NSP digestibility increased following enzyme addition, the birds did not benefit from this effect in terms of improvements in AME content and growth performance (Manuscript 2). As documented earlier, the lower gut fermentative capacity of the chicken is limited and most of the energy from NSP utilization is in the form of monomeric sugars (Moran, 1982). It is known that vegetable protein sources contain high levels of NSP. Therefore, appreciable improvements would be expected if effective enzymes are devcloped to degrade these components. Future research is needed to more precisely characterize the structure of various NSP present in vegetable proteins and to develop highly efficacious enzymes capable of degrading NSP to their constituent sugars which would lead to the utilization of both soluble and insoluble NSP as an energy source for poultry.

In the studies on full-fat canola and flax seeds (Manuscripts 4 and 5), it was found that fat digestibility did not reach the optimal level following enzyme supplementation of

the same enzyme blend even at the high level. It was also noted that the NSP digestibilities determined in both the TME_n assay and broiler chicken trial using flaxseed were higher than the values obtained from the TME_n assay and broiler chicken trial using canola seed. However, the TME_n content of flaxseed or the AME of flaxseed containing diets were much lower than those obtained from canola seed, even though there was no difference in energy availability between flax and canola oil (Lee et al., 1995). In this context, the viscous property of flax mucilage might have contributed to the lower energy availability of flaxseed when compared with canola seed. In order to achieve greater energy utilization from flaxseeds, further research is needed to develop enzyme combinations more effective in flax mucilage viscosity reduction.

It is also noted that there seems to exist an apparent differences observed with adult roosters and young broiler chickens with young broiler chickens showing much smaller response from enzyme supplementation (Manuscripts 4 and 5). This could be due to the fact that the concentration of oilseeds (15%) in the broiler diets was much smaller (15%) compared to the 100% of the single ingredient used in the TME assay. Therefore, the enzyme effects may be proportional to the concentration of the target ingredients. On the other hand, TME assay are often performed under fast conditions which could alter physico-chemical chyme features and/or physiological conditions (relationship between nutrient/energy supply and degree of meeting nutrient/energy requirement, degree of gut filling, etc.) in such a way that the effects of enzyme supplements could become more obvious. Marginal nutrient supply might provoke an increase in nutrient utilization whereas ad libitum feeding conditions in any growth experiment may result in relatively sufficient nutrient supply even in the control groups. This suggests that the TME assay

with adult roosters could serve as a fast means of enzyme evaluation while the real effect of enzyme supplementation should be evaluated with broiler chickens.

The benefits of using enzymes in poultry diets in the future should include not only enhanced growth performance and feed conversion, but also less environmental polution due to reduced output of excreta. Increased accuracy and flexibility in lease-cost feed formulations and improved well-being of animals are other possible benefits of enzyme use. It is possible that the digestibility of feed components can be tailored to produce end products with specific effect on the gut microflora and the immune system. Austin et al. (1999) reported that a cloned endo-1-4 xylanase produced the same range of oilosaccharides from different wheats fed to chickens. This means that a specific range of oligomers can be produced from a given NSP source in situ with a particular enzyme. Some of these carbohydrates may be used to stimulate the development of beneficial microflora in the gut. As an example, production of xylo-oligosaccharides by the use of xylanases in wheat-based diets has been indicated as one way to encourage the development a healthy gut microflora (Vahjen et al., 1998). The role enzymes can play in an antibiotic free production era is an area of great interest for future research.

9. CONCLUSIONS

1) In the in vitro incubation studies using wheat, SBM, CM, peas, and flaxseed meal, a more pronounced degradation of NSP was achieved when the enzyme preparations were used in concert;

2) The addition of combinations of carbohydrase enzymes could further improve enzyme efficacy in practical broiler diets based on wheat, SBM, canola meal, and peas;

3) The nutrient utilization of corn-SBM diet by broilers could be enhanced by using multicarbohydrase supplements;

4) The nutrient encapsulating effect of cell wall polysaccharides in SBM, CM, and peas may not be the only factor responsible for incomplete nutrient utilization;

5) The negative effects of soluble NSP on animal fat digestion are substantial even in a practical low-viscosity wheat-based broiler diet and multicarbohydrase preparations could eliminate the negative effects of soluble NSP on animal fat utilization;

6) A multicarbohydrase preparation was effective in improving energy utilization from full-fat canola and flax seeds and thus their feeding values for broiler chickens.

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