EFFECT OF A NEW MULTI-ENZYME SUPPLEMENT ON NUTRIENT DIGESTIBILITY AND GROWTH PERFORMANCE OF BROILER CHICKENS

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

Xinyu Zhou

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

Department of Animal Science

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

Effect of a New Multi-Enzyme Supplement on Nutrient Digestibility and Growth Performance of Broiler Chickens

BY

Xinyu Zhou

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree

of

Master of Science

ABSTRACT

The effect of a new enzyme blend on nutrient utilization and animal performance was investigated. The enzyme blend was developed to enhance the less than optimum utilization of certain feed components by monogastric animals through the use of in vitro experiments studying the effect of exogenous enzymes on hydrolysis and/or depolymerization of nonstarch polysaccharide (NSP), phytate, glycoprotein(s) and starch of wheat cell wall material, barley, canola meal and soybean meal. Enzyme compatibility and their potential synergistic effects were also examined. The most promising enzyme blend was validated in growth performance experiments with broiler chickens (from day 4 to day 18). The new enzyme blend (Enzyme B) was added to a diet moderately deficient in energy, protein and available P and composed of wheat (47%), hulless barley (11%), soybean (19%) and canola meal (11%). The Enzyme B supplemented diet was compared to a control (no enzyme) diet and a diet supplemented with conventional enzymes (ie., xylanase and β-glucanase)(Enzyme A). Relative to control, Enzyme A slightly improved body weight gain (BWG: 440 g vs. 447g) and significantly decreased feed conversion ratio (FCR: 1.57 vs. 1.54). Further improvement in body weight gain (460g) and FCR (1.51)($p<0.05$) was noted for Enzyme B. Significant enhancement in utilization of feed components was observed for the two enzyme supplemented diets with Enzyme B showing superior dry matter (67.8, 64.7, 61.0%), energy (AME_n)(2826, 2694, 2532 kcal/kg), starch (98.1, 95.2, 91.7%), phytate (33.2,

26.6, 26.0%) and NSP (20.7, 12.7, 3.7%) digestibility as compared to that of Enzyme A and the control diet, respectively. As expected, digesta viscosity was significantly reduced following Enzyme A and Enzyme B supplementation (ie., Control - 4.7cps; Enzyme A - 2.8cps; Enzyme B - 2.9cps). An improvement in broiler chicken performance, following Enzyme B supplementation, was confirmed in another trial in which a similar trend in BWG increase (385g vs 408g) and FCR reduction (1.56 vs 1.51) was observed. It would appear evident from this study that the application of an effective blend of enzymes could allow for the use of less expensive ingredients and more cost effective formulation of young poultry diets.

ACKNOWLEDGMENTS

The author wishes to express her sincere gratitude to her advisor Dr. B. Slominski for his guidance and encouragement towards the completion of this work.

I would like to thank my advisory committee, Drs. L. Campbell, S. Badoo and M. Scanlon for their constructive discussion and valuable advice.

The author would also like to thank the technical staff of the nutrition laboratory for their skillful technical help.

The scholarship from the Natural Science and Engineering Research Council of Canada (NSERC) and Canadian Bio-system Inc. is gratefully acknowledged.

Finally, I would like to express the greatest thank to my parents, husband and brother for their patience, support and sacrifice which the author is in deep debt.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

NSP: Non-starch polysaccharides

AMEn: Apparent metabolizable energy

FCR: Feed conversion ratio

SD: Standard deviation

GI: Gastrointestinal tract

CM: Canola meal

BWG: Body weight gain

GE: Gross energy

1. INTRODUCTION

Cereals, as high-energy ingredients, have been widely used in poultry diets for many years. Their utilization is limited to some extent by high fiber contents. It has been reported that the fiber components of barley, oats, rye and wheat can not be digested by the endogenous enzyme secretions of poultry. Cell wall or non-starch polysaccharides (NSP) of cereal grains are known to have an anti-nutritional effect on animal performance (Pettersson and Aman 1988,1989; GrootWassink et al., 1989). The problems observed with the feeding of barley, rye and oats have been associated with the presence of water-soluble β-glucan and arabinoxylan. These highly viscous polysaccharides increase viscosity in the small intestine of the chick, decrease digestibility and absorption of nutrients, produce sticky droppings and consequently depress the growth of the chicken.

In contrast to barley, oats and rye which are known to be of lower value for poultry, wheat is considered a superior feed ingredient, although its feeding value can be highly variable. Considerable research data demonstrated large variability in the apparent metabolizable energy content of wheat (AME) (Mollah et al., 1983; Rogel et al., 1987), and that the AME values are negatively correlated with the content of water-soluble NSP (Annison, 1991; Choct and Annison, 1990, 1992).

The anti-nutritive properties of cereal grains can be effectively overcome by the addition of enzyme preparations containing endo-β-glucanase and xylanase (Gohl et al.,

1978; Hesselman and Aman, 1986; Pettersson and Aman, 1988; Classen et al., 1988; GrootWassink et al., 1989; Rotter et al., 1989a,b,c; Pettersson et al., 1991; Bedford et al., 1991; Friesen et al., 1992; Annison, 1991; Boros et al., 1993). The improvement in performance following enzyme supplementation has been reported to be due to the breakdown of arabinoxylans (pentosans) and β-glucans into smaller polymers (De Silva et al., 1983) which alter the ability of these polysaccharides to form highly viscous solutions. The disruption of the intact cell walls and release of nutrients encapsulated within the cells has been also given serious consideration (Hesselman and Aman, 1986).

Although many beneficial effects have been reported with the use of enzymes in cereal-based diets, highly variable responses to enzyme supplementation in wheat-based diets have been reported. Research conducted at the University of Manitoba on a number of Canadian wheat and hulless barley samples has shown that supplementation of barley-based diets with β-glucanase and wheat-based diets with xylanase resulted in less pronounced response than when both enzyme activities were used in concert. Therefore, a study on the mechanism by which exogenous enzymes function in the bird's gastrointestinal tract has been undertaken. To date, only a few in vitro experiments have been conducted to determine the effect of different cell-wall-degrading enzymes on cell wall polysaccharide depolymerization and release of nutrients entrapped inside the cells (Mulder et al., 1991). No research data, that the author is aware of, exist on compatibility of various carbohydrase, protease or amylase enzymes and the effect may have on chick performance.

In addition, very little is known on the potentially beneficial effects of enzymes which could be used to augment the animal's own digestive system. In this context, the

young animals are known to produce insufficient amounts of some enzyme activities (i.e., amylase, protease, lipase) (Noy and Sklan, 1995,1997; Nitsan et al., 1991; Jin et al., 1998;)

Therefore, the main purpose of the present study was to develop a cocktail of exogenous enzymes to improve nutrient utilization by young animals with immature digestive system and insufficient production of endogenous enzymes. The research conducted over the last two years included: (1) in vitro evaluation of a wide range of enzyme combinations for their ability to hydrolyze the cell wall structure and to release nutrients (ie., protein, starch) encapsulated within the cells, and (2) in vivo studies on the effect of different enzyme blends on nutrient utilization and broiler chicken performance. Furthermore, utilizing some of the knowledge on use of enzymes in cereal-based diets, the effect of specifically designed enzymes to improve the nutritive value of canola meal and soybean meal was also investigated.

2. LITERATURE REVIEW

2.1. Introduction

The use of selective multi-enzyme preparations in the animal-feed industry is just in its beginning. Since the first experiments in 1957 (Jensen et al. 1957), the market has grown rapidly with increased understanding of enzymes and their properties. Now the total worldwide market value of feed enzymes has grown to an estimated 60 million US$ in 1995 and is expected to double in value by the year 2005 (Hepner, 1995). The widespread use of enzymes in animal feeds is a reflection of changing altitudes in society and the economic climate of the feed industry. Consumer's attitudes toward a drug-free, natural food supply promote such products as enzyme and direct feed microbials as additives. Another advantage of the application of enzymes is that it allows producers to reduce the costs of feed formulation by the use of a wider range of ingredients currently considered inferior such as barley, wheat, oats, rye, canola meal or peas.

For commercial diet formulation, the efficiency of utilization of wheat, oats, barley and rye by poultry is often limited by the presence of certain fibre components. Most of the dietary fibre in cereals are nonstarch polysaccharides (NSP) in which the major cell-wall polysaccharides of concern are the β-glucans in barley and oats and the arabinoxylans in rye, wheat and triticale. These fractions cannot be digested by the endogenous enzymes of poultry and consequently have antinutritive effects. Numerous

studies have demonstrated that water-soluble NSPs, not the total NSPs, cause highly viscous conditions in the small intestine of poultry (Classen and Bedford, 1991). The increased intestinal viscosity is known to reduce the rate of nutrient diffusion (Fengler and Marquardt, 1988a,b) which increases microbial population in the small intestine (Feighner and Dashkevicz, 1988) and adversely affects nutrient utilization (White et al., 1983; Hesselman and Aman, 1986). The β-glucans and arabinoxylans that form the endosperm and aleurone layer walls of cereals hinder nutrient digestion through an encapsulating effect of the cell walls. This, in turn, prevents the animal's own digestive enzymes from accessing the nutrients contained within the cells (Hasselman and Aman, 1986; Petterson and Aman, 1989).

Supplementation of exogenous enzymes has proved to be beneficial to poultry for many years. It has been well documented that exogenous enzymes have potential to destroy the gel-forming polysaccharides, reduce intestinal viscosity and promote substrate-enzyme interaction which increases nutrient availability (Friesen et al., 1992). In addition, exogenous enzymes can disrupt the intact cell walls of cereals releasing entrapped nutrients, rendering certain nutrients biologically more available and making fibre components more available as an energy source (Petterson and Aman, 1988; Bedford et al., 1991; Bedford and Classen, 1992;Van Paridon et al., 1992). Most of the specific enzymes used in cereal-based diets for poultry are xylanases for wheat, triticale and rye and β-glucanases for barley and oats.

Utilizing the knowledge on enzyme use in cereal-based diets, the application of enzymes for improving the nutritive value of corn/soybean and canola meal-based diets has been under investigation. The addition of enzyme preparation to soybean/corn diets

or pre-treatment of such feedstuffs with enzymes have been reported to improve nutrient utilization (Classen et al., 1993; Meijer et al.,1995) and broiler chicken performance (Silersides and Bedford, 1999). However, the results of various feeding trials have not been consistent. The use of canola meal as a protein source in diets is limited by its relatively high fibre content. Research from this laboratory has shown that dietary fibre components can be degraded by exogenous enzymes and that the utilization of canola meal could be improved (Slominski et al., 1992; Simbaya et al., 1996).

Young chickens, especially during the first few weeks, are known to produce insufficient amounts of some enzymes, i.e. amlyase, protease, lipase (Nitsan et al., 1991; Uni et al., 1995; Noy and Sklan, 1995). Therefore, dietary nutrients such as fat, protein and energy are poorly utilized because of the immaturity of the digestive system (Jin et al., 1998). Supplying enzymes lacking in the endogenous secretions offers the opportunity for increased nutrient utilization. Such preparations include α-amylase to improve starch digestibility or protease to degrade protein and improve the availability of amino acids.

This review brings together the information on the effect of dietary enzymes on nutrient utilization and animal performance when fed common cereal grains and oil-seed meals. The enzyme addition to augment the young animal's digestive capability will also be discussed.

2.2. What are enzymes

Biologically, enzymes are functional proteins that catalyze or accelerate the rate of specific chemical reactions. Most enzymes associated with digestion are hydrolytic. They are classified by the substrates upon which they react. Table 1 illustrates the classifications of enzymes used by the animal feed industry.

Most commercial enzymes used today are of bacterial (*Bacillus sp.*) or fungal (*Aspergillus sp.*) origin. Although the current-generation enzymes are highly beneficial, their usefulness is still limited. Their stability under the heat and moisture conditions encountered in feed processing is of concern to feed manufactures. Under practical feed mill conditions (i.e., pelleting), the enzymes are stable up to 75°C whereas significant inactivation occurs at 95°C (Inborr and Bedford, 1994). Furthermore, once the enzymes enter the gastrointestinal tract, they are subjected to conditions like low pH and proteolytic enzyme activities in the proventriculus and gizzard, which may cause enzyme denaturation and result in their reduced activity.

In order to achieve a positive effect, feed enzymes must remain active within the digestive tract. Most feed enzymes of bacterial origin exhibit pH optima close to neutrality while fungal enzymes generally exhibit maximal activity under more acidic conditions (pH 4.0-6.0). Therefore, a mixture of enzymes from both fungal and bacterial sources is recommended for the animal-feed industry to ensure activity over as broad a pH range as possible.

Table 1: General enzyme classifications categorized by substrate functionality

SUBSTRATE	ENZYME EXAMPLE
Protein	Protease, Peptidase,
Starch	Amylase
Lipids	Lipase
Cellulose	Cellulase, Cellobiase
Arabinoxylans (wheat, rye)	Xylanase
β-glucans (barley, oats)	β-Glucanase
Pectins	Pectinase
Mannans, Galactans, Arabinans	Mannanase, α-Galactosidase
Phytate	Phytase
Galactooligosaccharides	α-Galactosidase

Source: Ferket, 1993 (modified)

Other means of overcoming enzyme stability problems would be to develop new enzyme preparations that are heat-stable and of higher activity toward indigestible components of animal feeds. Advancements in genetic engineering make this possible. Alternative sources of enzyme have also been studied. Enzymes may not only be produced directly from fungi and bacteria but also from plants or could be expressed in microorganisms in the rumen of cattle.

2.3. Addition of enzymes to cereal-based diets for broilers

The use of oats, barley, rye, and to some extent wheat as energy sources has been limited in monogastric feed regimens, especially in starter diets for young animals. In comparison with corn, all these cereals have relatively high fiber contents. The high fibre content has been implicated as the major negative factor relating to poor nutrient utilization (Moran et al.,1969; Fengler et al.,1988a,b; Heger et al.,1990; White et al.,1983). Addition of enzyme to diets has proven to increase nutrient digestibility and bird performance (Moran et al. 1969; Pettersson and Aman, 1988,1989; GrootWassink et al., 1989). In order to increase the potential for enzymes to be more effective, a better understanding of the grain or seed structure, chemical composition, nutrient digestibility and the anti-nutritive properties of various dietary components is necessary.

In most feedstuffs (Table 2), dietary fiber components (i.e., non-starch polysaccharides, glycoproteins), phytate, oligosaccharides or some fractions of starch and protein undergo a limited conversion to substrates available for absorption and could be

Table 2. Chemical composition of most commonly used feedstuffs (%, as fed basis)

Component	Wheat	Corn	Barley	Soybean meal	Canola meal	Peas
Protein	12.8	8.5	11.0	44.0	36.0	21.9
Sugars	1.2	1.5	1.5	7.2	6.7	3.6
Oligosaccharides	1.2	0.3	0.8	6.0	2.5	4.8
Starch	59.0	64.1	53.6	1.8	2.0	41.1
Total Fiber:	11.2	8.7	18.6	22.4	30.1	18.6
- Polysaccharides	9.5	6.9	15.1	19.1	18.0	14.8
- Lignin & others	1.7	1.8	3.5	3.3	12.1	3.6
Phytate	1.2	0.7	1.3	1.5	2.8	0.8
Ash	1.9	1.4	2.6	5.8	7.0	2.6
Fat	1.7	3.8	1.9	2.2	4.0	1.5
Total	89.8	88.7	90.0	89.4	87.8	91.3

Source: Slominski, B.A., University of Manitoba

degradable by exogenous enzymes of microbial or fungal origin, thereby, improving the nutritive worth of such feed ingredients.

2.3.1. Grain structure

In cereal grains, the most important nutrient is starch composing 50-70% of the total weight (Table 2). Starch is located in the endosperm. The endosperm is enclosed by a thin layer of the cells known as the aleurone layer with relatively thick cell walls.

Biologically, the plant cell wall is a complex structure in which microfibrill of cellulose form a rigid, highly ordered skeleton. The skeleton is embedded in a gel-like matrix which is less ordered and composed of the non-cellulosic polysaccharides and glycoproteins (Samman and Annison, 1993). The nature of the cellulose microfibrills varies among plants. The types and levels of the polysaccharides of the amorphous matrix show considerable differences among species. In cereals, the cell wall polysaccharides are mainly composed of arabinoxylan, β-glucan and cellulose. In wheat and rye, arabinoxylans predominate whereas in barley β-glucans are the main components. All these plant structural polysaccharides (NSPs) are the major components of dietary fibre that is resistant to hydrolysis by the alimentary enzymes of animal origin (Trowell et al., 1976). Cellulose constitutes a small proportion of the cereal cell walls (Table 4) and is thought to be of litter nutritional consequence as it is for the most part water-insoluble. β-glucan is a polymer of glucose residues linked via β-1,4 linkages, interspersed by a single β-1,3 linkages (Henry,1987). The presence of the β-1,3 linkages which differentiates β-glucan from cellullose results in the polymer being more soluble and viscous in solution.

The overall solubility of β-glucan relates to the degree of association of the glucan with insoluble cell wall fractions (Classen and Bedford, 1991). The chemical structure of β-glucan is shown in Figure 1.

Arabinoxylan (pentosan), unlike cellulose or β-glucan, is a heteropolymer consisting of a backbone of β-1,4-linked xylopyranosyl residues with terminal 1,2 and 1,3 arabinofuranosyl substitutions (Figure 2). Arabinofuranosyl substitution reduces the ability for hydrogen bonding between carbohydrate chains and consequently results in fractions which are water-soluble and highly viscous (Classen and Bedford, 1991). The total and water-soluble arabinoxylan and β-glucan contents of cereal grains are shown in Table 3. It is difficult to differentiate between soluble and insoluble arabinoxylan as it is affected by the degree of branching and the molecular weight. In addition, the proportions of major polysaccharides present in the endosperm and the aleurone cell walls are different (Table 4). The structural protein levels also differ as wheat endosperm cell walls contain 4.7% of the total protein of the grain while the aleurone cell walls have as high as 10.5% of wall-inserted protein (Bacic and Stone, 1981). It has been shown that arabinoxylan found in the aleurone cell walls is less water-soluble than that present in the starchy endosperm (Henry, 1985).

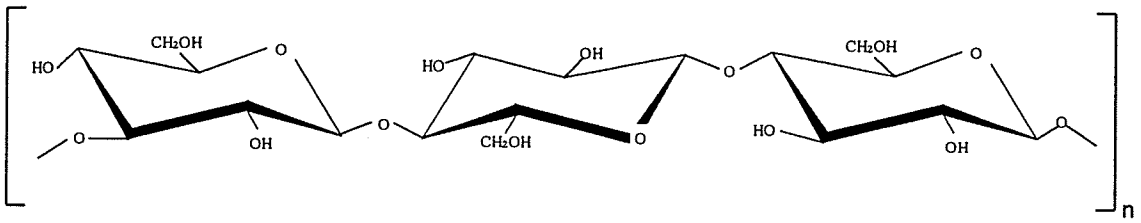

Figure 1. Chemical structure of β-glucan (Fincher and Stone, 1986)

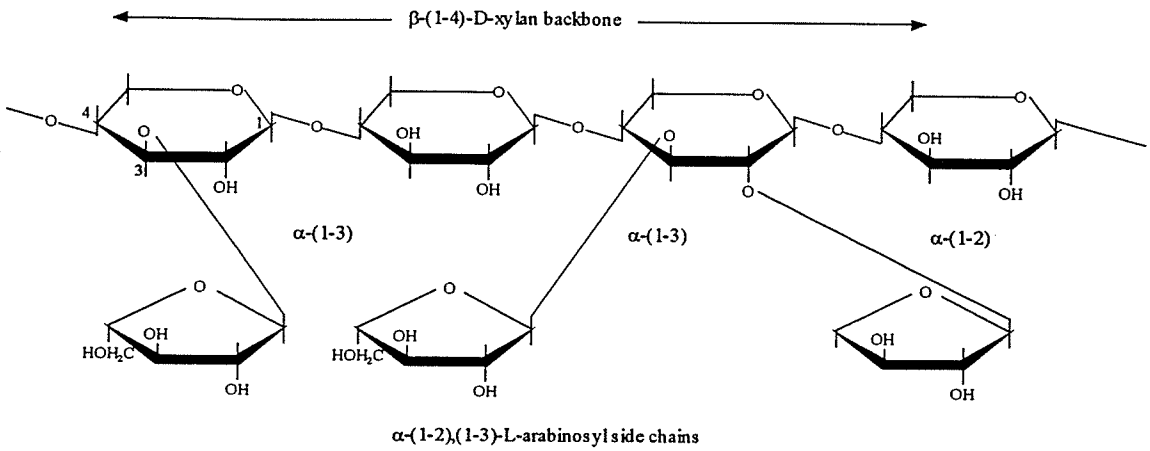

Figure 2. Structure of arabinoxylan {α-(1→2,3)-L-arabinofuranosyl-}-β-(1→4)-D-xylopyranan (Fincher and Stone, 1986)

Table 3. The total and water-soluble arabinoxylan and β-glucan contents of cereal grains (g/kg DM)

Cereal	Arabinoxylan		β- Glucan	
	Total	Soluble	Total	Soluble
Barley	56.9	4.8	43.6	28.9
Oats	76.5	5.0	33.7	21.3
Rye	84.9	26.0	18.9	6.8
Wheat	66.3	11.8	6.5	5.2

Source: Henry (1987)

Table 4. Protein and polysaccharide content of the aleurone and endosperm cell walls:

	Protein (%)	Major polysaccharides
Starchy endosperm	4.7	Arabinoxylan (70%) (1-3,1-4)-β-Glucan (20%) Cellulose (4%) Glucomannan (7%)
Aleurone layer	10.5	Arabinoxylan (65%) (1-3,1-4)-β-Glucan (29%) (1-3)-β-Glucan (1%) Cellulose (2%) Glucomannan (2%)

Source: Bacic and Stone (1981); Fincher and Stone (1986)

2.3.2. Anti-nutritive properties of NSPs

Methods of improving the performance and hence the economic value of barley, rye, oats or wheat used in diets for poultry have been studied for many years. Feed processing techniques, like pelleting and extrusion, result in increased nutrient digestibility and improved feed efficiency in birds because of starch gelatinization and disruption of the endosperm cell walls (Tovar et al., 1991). However, the intestinal viscosity in birds fed such treated diets has been shown to increase as the soluble dietary fibre increases (Teitge et al., 1991; McCracken, et al., 1993). Dehulled grain contains 10-15% less crude fibre as compared to the hulled grain. In this regard, the advent of hulless grain through plant breeding was expected to produce a feed with increased digestible nutrient content because of the removal of the indigestible fiber fraction of the pericarp. However, several experiments with hulless barley have shown that this grain in fact led to an exacerbation of some of the problems (Classen et al., 1985). Therefore, more research has been focused on the arabinoxylans and β-glucans in the endosperm of barley, rather than on the pericarp components of the hull fraction.

Barley β-glucan

Experiments conducted by Bedford and Classen (1992b) demonstrated that feeding a 60% barley-based diet to broiler chickens from 0 to 3 weeks of age resulted in significantly increased intestinal viscosity and decreased performance. When isolated β-glucan from barley was added back to a broiler diet (White et al., 1981), a depression in growth performance as well as an increase in gut viscosity was noticed. This was confirmed in another study by White et al. (1983).

Rye and wheat arabinoxylan

Rye has not been used widely as an energy source in poultry diets because of its high pentosan (arabinoxylan) content (8.5% of dry matter) (Henry, 1987). When, in one study, 1.3% water-soluble pentosans isolated from rye were added to a wheat-based diet, the same growth depressing effect in chicks was observed as that from a 56% rye-based diet. This depressed performance was related to increased digesta viscosity and the production of sticky droppings. The water-soluble pentosans have been indicated to be responsible for the increased viscosity and anti-nutritive effects (Antoniou and Marquardt, 1981; Ward and Marquardt, 1987; Fengler and Marquardt, 1988a).

Significant variability in the AME content of wheat has been reported with values ranging from 11 to 13 MJ/kg dry matter (Mollah et al., 1983). To assess if the pentosans in wheat (ie., 5.7-8.2% of total) were responsible for the low-ME wheat phenomenon, two major studies were conducted by Rogel et al. (1987) and Annison (1991). The studies summarized the relationship between the contents of pentosan and AME values in various wheat varieties grown in Australia and indicated that there was no relationship between wheat total pentosan and AME (Table 5). However, isolated water-extractable NSPs consisting primarily of pentosan were shown to be closely correlated with the low AME content (Choct and Annison, 1990; Choct and Annison, 1991; Annison, 1991). Parallel to the depression in growth and AME content of the diet, the reduction in starch, protein, and lipid digestibility was noted (Choct and Annison, 1990). It has been concluded that wheat pentosans act in the same manner as the arabinoxylans of rye or β-glucans of barley (Annison and Choct, 1991)

Table 5. Relationship between AME and total pentosan content of various wheat varieties

Variety	AME (MJ/kg DM)	Total pentosan (%)
Owlet	11.25 ± 0.64	6.95
Sunfiled	13.59 ± 0.54	6.71
Vulan	13.00 ± 0.83	6.35
Hartog	12.24 ± 0.52	7.00
Owlet	13.45 ± 0.45	6.29
Oxley	11.90	5.71
Kite	14.23	7.65

Source: Annison (1991) and Rogel et al. (1987)

The mechanism by which the pentosan or β-glucan exhibit their anti-nutritive effect is unclear. However, there appears to be a high correlation between the amount of soluble pentosans or β-glucans in cereals and extract viscosity (White et al. 1981; Antoniou et al., 1981; Antoniou and Marquardt, 1982; Dusel et al., 1998). When purified pentosan or β-glucan were fed to broiler chickens, digesta viscosity in the small intestine increased proportionally to the increase in polysaccharide content, and body weight gain and feed conversion ratio (FCR) decreased as intestinal viscosity increased (Bedford and Classen, 1992) (Fig. 3). Thus, it is well documented that the anti-nutritive activity of arabinoxylans and β-glucans in chickens is not a function of the polymers *per se*, but a consequence of the intestinal viscosity that they create (Bedford, 1993)

The exact effect of viscosity has not been established but a possible mechanism includes reduced rate of diffusion of endogenous enzymes in digesta and their impaired interaction with substrates (Fengler and Marquardt, 1988b). This could have an adverse effect on the rate of enzymatic degradation of ingested nutrient (Ikeda and Kusano, 1983) and a reduction in the digesta passage time. A reduction in the digesta flow rate could also result from increased microbial population in the small intestine (Salih et al., 1991). Intestinal microflora will digest and utilize the starch and protein in the digesta and would effectively compete with the host for nutrients. This further exacerbates the situation for the host (Bedford, 1995).

There is some disagreement as to the mechanisms by which the viscous polysaccharides in wheat exhibit an anti-nutritive effect. Although the endosperm walls of wheat contain arabinoxylan and β-glucan (up to 9.5% of dry matter), the solubility of these polysaccharides is lower in wheat than in barley and consequently their presence is

not always associated with an increased intestinal viscosity and incidence of sticky droppings. Data show that the range of foregut viscosity was from 1.5 to 21.1 cps (centipoids) and the range of hindgut viscosity was from 2.7 to 39.2 cps for broilers fed wheat diets under various conditions (Teitge et al. 1991; Veldman and Vahl, 1994; Bedford 1997; Allen et al. 1996; Dusel et al.1998). As shown by Steenfeldt et al. (1998), even at the high level of wheat (>80%) in the diet, digesta viscosity was 5 times lower than the viscosity values for birds fed diets with a high content of rye or barley (Bedford and Classen, 1992). The FCR and respective digesta viscosity values for poultry fed wheat were summarized by Liang and Liu (1998) and a correlation coefficient between the viscosity values and FCR was determined as low as –0.39. The data indicated that digesta viscosity is not a major parameter for prediction of broiler chicken performance when fed wheat-based diets (Liang and Liu, 1998). In other experiments conducted by Veldman and Vahl (1994), no difference in intestinal viscosity of broiler chickens fed diets containing wheat with high or low water-soluble arabinoxylan contents was found. These experiments questioned the hypothesis of the viscosity impact on the performance of broiler chicken fed wheat-based diet. However, recent experiments with xylanase addition to wheat-based diets indicated that the viscosity of digesta was still a factor resulting in poor performance. From a review of the literature data, Bedford and Morgan (1996) concluded that viscosity is a major factor responsible for the depressed performance of chickens fed wheat-based diets. Further work is needed to elucidate the factor involved.

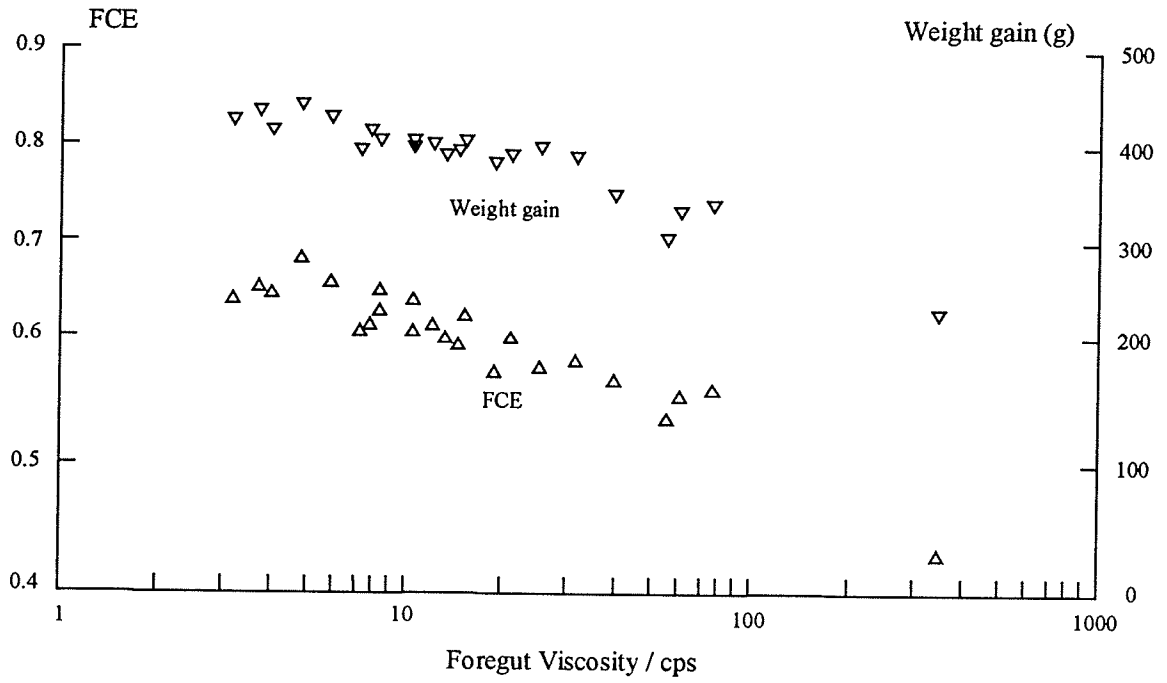

Figure 3. Effect of gut viscosity on weight gain and feed conversion ration (FCE)

(Bedford,1992)

2.3.3. Effects of enzyme use

The beneficial effects of enzyme addition to diets containing barley for poultry has been known for many years (Gohl et al., 1978; Classen et al., 1988). It is believed that the majority of the enzyme-induced improvement in the feeding value of barley is derived from the endo-β-glucanase activity (Ricks et al., 1962). In a study by Hesselman and Aman (1986), addition of β-glucanase to barley-based diets reduced viscosity of the intestinal contents from 28.5 to 2.9 cps, and consistently improved weight gains (from 349.7 to 420.7 g at the first 21 days) and feed conversion ratio (from 2.06 to 1.82) in broiler chickens. In addition, the side enzyme activities (i.e. xylanase, cellulase) present in the conventional enzyme preparations may also be involved in the improvement in the nutritive value of barley (Mulder et al., 1991).

For growing chicks, diets containing high levels of rye usually cause severe problems attributable to water-soluble, highly viscous NSPs (Campbell et al., 1983; Fengler and Marquardt 1988; Friesen et al.1992; Marquardt et al. 1994). The negative effects of the NSPs (pentosans) on the utilization of rye-based diets can be overcome to a considerable degree by adding crude-enzyme preparations with high endo-xylanase activity. A dose response study utilizing different levels of rye with or without xylanase confirmed that addition of enzyme significantly reduced intestinal viscosity and improved weight gain and FCE at each level of rye (Bedford and Classen, 1992). Despite the better performance obtained in broiler chickens fed an enzyme-supplemented rye-based diet, the practical use of rye in broiler diets is limited because of wet litter and management problems.

Many experiments have demonstrated that supplementation of wheat-based diet with xylanase improve AME values and bird performance (Annison, 1992; Petterson and Aman, 1992b; Van Paridon et al., 1992). Most of the increased performance was achieved by reducing viscosity, which confirms that wheat pentosans elicit anti-nutritive activity predominantly by increasing the viscosity of digesta in broiler chickens. However, literature data suggest that the success of xylanase supplementation is not as dramatic in wheat-based diets as in rye-based diets. In many studies, variable response to enzyme supplementation in wheat-based broiler diets has been reported and often no quality improvements were noted (Crouch et al., 1997). The inconsistencies may be attributable to variation in the chemical composition of different batches of wheat.

In studies on Australian wheats, chemical analysis of wheat samples showed high variability in quality (Mollah et al., 1983). The high-quality wheat contained more protein and starch and less fibre in comparison with the low-quality wheat. In order to study if enzyme effectiveness would be modified by source of wheat when used in the starter rations of chicks, low- and high-quality wheat were substituted into the diets with and without enzyme supplementation. Results showed that the digesta viscosity was reduced by addition of enzyme, but the increase in feed conversion ratio (FCR) was only observed for the low-quality wheat with added enzyme (control=1.49, control + enzyme = 1.33; $P<0.05$). Birds fed a high-quality wheat diet with enzyme had similar performance (FCR) which was comparable to that of high-quality wheat without enzyme (1.31 vs. 1.30) (Crouch et al., 1997). The study of Dusel (1998) also indicated that the source of wheat influenced the effect of enzyme addition and the high-quality wheat did not need the enzyme supplement to improve performance. All these data suggested that

the effectiveness of an enzyme preparation is highly dependent on the characteristics of the wheat variety.

The effect of enzyme supplementation using different amounts of wheat in the diets was examined by Steenfeldt et al. (1998). Experiments were conducted in which 633, 721 and more than 810 g/kg wheat with and without cell wall degrading enzymes were used. Weight gain and FCR increased by 5% and 6%, respectively when compared to the control at the inclusion levels of wheat in the diets up to 80g/kg. At this level the viscosity of intestinal content was in general reduced with enzyme supplementation (19.2 vs. 5.1 cps). However, with the first two inclusion levels of wheat (i.e., 63 and 72 g/kg), weight gain and feed intake were not significantly improved by enzyme addition. Veldman and Vahl (1998) also demonstrated that the effect of xylanase supplementation was influenced by the content of wheat in the diet and concluded that the effect can be explained partly by the degree of reduction of intestinal viscosity.

In contrast to the results discussed above, some recent studies have, however, shown that the response from enzyme supplementation as determined by AME (apparent metabolizable energy) content and bird performance is not always associated with decreased digesta viscosity. Cowan et al. (1994) reported that the intestinal viscosity is often between 10 and 15 cps with wheat-based diets and indicated that at low viscosity level (< 10 cps) further reductions in viscosity do not result in performance gains. Choct and Annison (1990) demonstrated that when a depolymerised pentosan preparation was added to a broiler diet, the performance of chickens (FCR) was not detrimentally affected when compared to the wheat-based control diet (1.59 vs. 1.65, $P< 0.01$), although the digesta viscosity from the diet containing the depolymerised pentosan was significantly

higher than that of the control. This suggests birds can tolerate small increases in the digesta viscosity without a significant effect on performance.

Thus, the exact mode of action of xylanase is still not fully understood. Although in many cases the benefits from xylanase addition to wheat-based diets can be explained partly by extract viscosity and the soluble polysaccharide fraction, other possible modes of action may play a role.

2.4. Addition of enzymes to corn/soybean and canola meal diets

Until recently, it has been assumed that corn and soybean are not of major concern with regard to digestive problems in poultry. Because of the lower amounts of water-soluble NSPs, these feedstuffs are less susceptible to improvement by the addition of dietary enzymes (Marquardt et al., 1994).

However, contrary to the common assumption that corn is relatively constant from batch to batch, a recent study has showed that its energy content varies considerably due to the harvesting condition (2,926 to 3,474 kcal ME/kg) (Leeson et al., 1993; Baidoo et al. 1991). There is also evidence to suggest that a significant proportion of the starch in the corn endosperm remains undigested within the chick ileum. As documented by Noy and Sklan (1995), the digestibility of starch in corn was found to be as low as 82%. Variability was also found in soybean meal samples in terms of quality and the levels of antinutritional factors such as trypsin inhibitors and lectins.

Based on these variations, a mixture of xylanase, protease and amylase was shown to improve metabolizable energy (3,076 vs. 3,153 kcal/kg), protein digestibility (80.0 vs.

82.9%) as well as broiler chicken performance (FCR) (1.86 vs. 1.82) of corn/soybean meal-based diets (Silersides and Bedford, 1999). In addition, enzyme addition was found to influence the digestibility of amino acids. The digestibilities of aspartic acid and threonine, which are known to be high in endogenous secretions, were improved significantly with enzyme addition while those of methionine and histidine, known to be low in endogenous secretions were not (Zanella et al.,1999). This suggests that the advantage of enzyme addition to corn/soy diets is not the direct result of improved digestibility of the feed. Rather, it is the result of a reduction in the endogenous protein losses from the digestive tract of the bird. In another experiment, a complex of glucanase, galactosidase and protease was found to be effective in improving the nutritive value of corn/soy diets for broiler chickens (FCR: 2.06 vs. 1.91, $P< 0.05$)(Zatari and Ferket, 1990). Scheideler et al. (1992) demonstrated that broiler chickens fed corn/soy diets supplemented with phytase had improved body weight gain, feed conversion and livability (Table 6). Trials have also confirmed that pre-treatment of soybean meal with protease and α-galactosidase can degrade soy trypsin inhibitors and increase N retention ($P<0.01$) and true metabolizable energy (Ghazi et al., 1996a,b). All these data indicate that the addition of a proper enzyme preparation can improve nutrient utilization (Pack et al., 1997,1998) and broiler chicken performance (Silersides and Bedford, 1999) when fed corn/soy diet. However, the results of various other feeding trials were not wholly consistent (Iridh et al., 1995; Knap et al., 1995). Further research is needed to clarify the role of exogenous enzymes in a corn/soy diet.

Table 6. Effect of phytase supplementation on broiler chicken performance when fed a corn/soy diet

Parameter	Control	+ Phytase	P< value
Body weight at 9 weeks (kg)	2.88	2.96	0.04
Feed/Gain 0-9 weeks	2.36	2.27	0.007
Livability (%)	74.8	82.1	0.004

Source: Scheideler et al. (1992)

Canola meal (CM) contains high quality protein but its use in diets of monogastric animals has been limited by the relatively high level of fibre (Table 2)(Bell, 1993), resulting in low energy yield and less than optimum protein utilization. Research from this laboratory has shown that with the addition of a dietary cell-wall degrading enzyme supplement to a laying hen diet containing 40% commercial CM, the NSP apparent digestibility was increased to 37%, in comparison to 2.3% for the control diet (Table 7) (Slominski and Campbell, 1990). Protease, carbohydrase and phytase enzymes, when added individually into a canola meal diet, showed 3%, 10% and 7% improvement in chick growth and 2%, 5% and 4% improvement in FCR, respectively (Guenter et al., 1995). Further studies on canola meal were conducted by Simbaya et al. (1996). Enzyme supplementation improved broiler chicken weight gain and FCR by 6.0% and 7.2%, respectively ($P<0.05$). In another enzyme evaluation study, canola meal was subjected to enzyme treatment with cellulase, α-galactosidase and phytase enzymes (Slominski et al., 1999a). The enzyme-treated meal resulted in improved weight gain (109.5 vs. 116.9g) and FCR (2.97 vs. 2.79) in weeks 3 and 4 of a rat growth trial. A significant enhancement in total NSP (1.4 vs. 9.8%), soluble NSP (19.8 vs. 28.0%), oligosaccharides (61.3 vs. 100%) and phytate (31.1 vs. 97.7%) digestibilities was observed for the enzyme-treated meal. Thus, it is believed that many components of canola meal could be degraded by proper exogenous enzymes of microbial or fungal origin, thereby, improving the nutritive worth of the meal.

Table 7. Digestibility of NSP in laying hens fed 40% canola meal with and without added enzyme (%)

Treatments	Polysaccharides		
	Total	Cellulose	Non-cellulosic polysaccharides
Canola meal	2.3	0.1	3.2
Canola meal + enzyme	36.6	13.0	40.5

Source: Slominski and Campbell (1990)

2.5. Addition of enzyme to augment the digestive capability of young animals

From the time of hatch, the gastrointestinal (GI) tract of the chicken is in a process of development and maturation. With the development of the GI tract and the pancreas, the concentrations of all enzymes in the pancreas increase at different rates. Research conducted by Nitsan et al. (1991) demonstrated the low specific activities of trypsin, amylase and lipase during the first few days after hatching. This low enzyme activity is because of the decreased reserves of pancreatic enzymes that are produced during embryonic growth. On days 11, 14 and 21 the specific activity of trypsin, amylase and lipase increased progressively and became approximately 15% higher than that at hatching (Figure 4). In another experiment, insufficient amylase activity in pancreas was also noticed at hatching (Krogdahl and Sell, 1989) and was approximately 20% lower than the activity observed at 56 days of age.

Due to insufficient enzymatic activity at the first few days after hatching, it was not surprising that the small intestinal digestibility of fatty acids was only 85% at 4 days of age (Uni et al., 1995), then slightly increased to over 90% in 12-15 day old chickens (Renner and Hill, 1960). In contrast to the report by Marchain and Kulka (1967) who demonstrated that chickens are fully capable of digesting starch shortly after hatching, incomplete starch digestibility, i.e., 85% and 90-98% was reported by Noy and Sklan (1995) and by Uni et al.(1995), respectively. Nitrogen digestion, reported by Noy and Sklan (1995), increased from 77.8% on day 4 to nearly 90% on day 21.

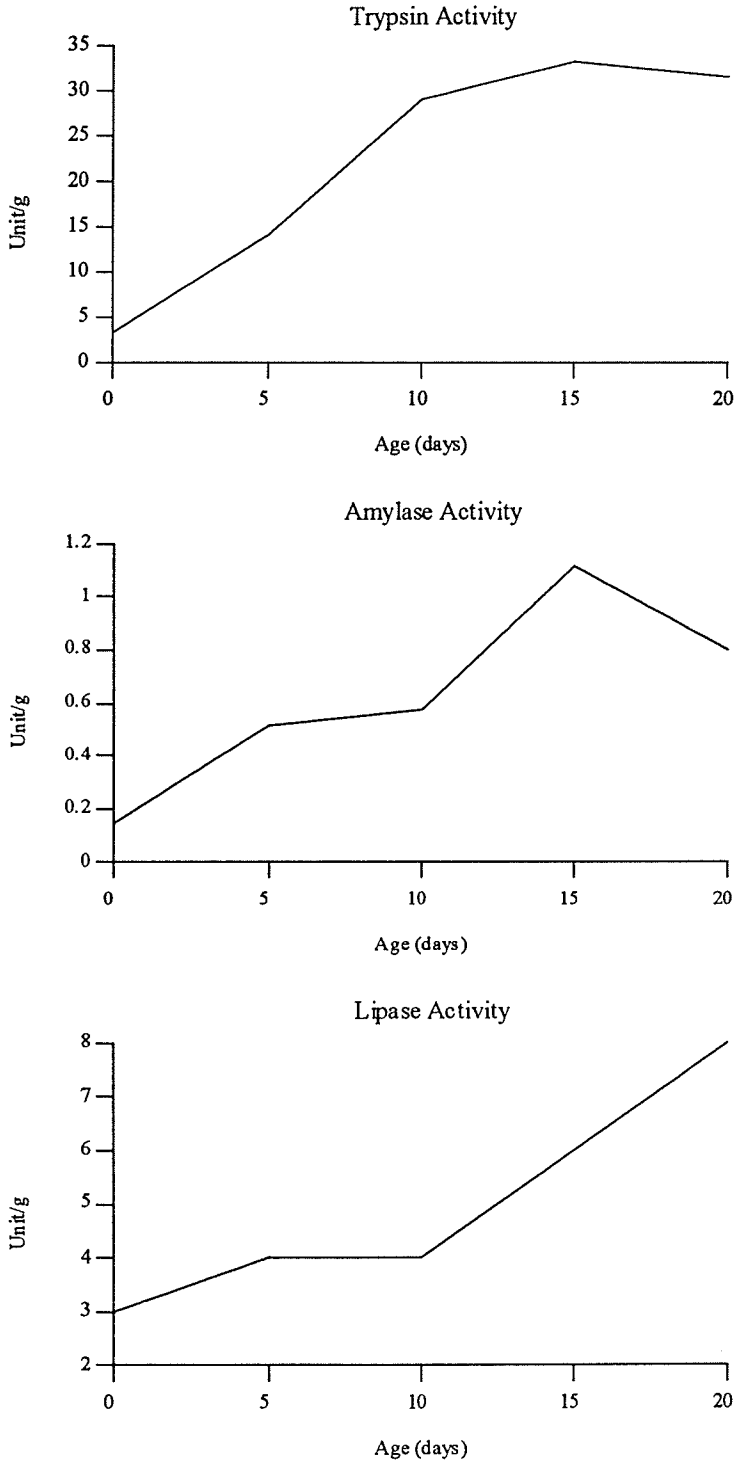

Figure 4. Activities of trypsin, amylase and lipase in the pancreas of male chicks from hatching to 23 days of age. (Nitsan *et al.*, 1991)

the increased nutrient utilization indicated that the total pancreatic enzyme activity may not be sufficient in the early post-hatch period for effective utilization of nutrients.

Based on the fact of limited production of digestive enzymes and incomplete nutrient utilization by young poultry, supplementation of the animal's own digestive system by specific exogenous enzymes have been under investigation. Enzyme supplements of starter feeds containing amylases, proteases and lipase have been reported to improve feed utilization (Collier and Hardy, 1986b; Parkany-Gyarfas and Toth, 1978).

2.6. The need for a multi-enzyme preparation

Enzymes have the greatest potential in diets that contain antinutritional factors that hinder nutrient availability. The cell wall polysaccharides in feedstuffs are the main target substrates for commercial enzymes. The hemicelluloses and pectins in protein supplements such as soybean meal and canola meal. are of concern. Therefore, each of feedstuff requires an enzyme specific to its polysaccharide component.

For cereals, most of the research data support the concept that the increased viscosity is the major factor responsible for the decrease in nutrient utilization. The NSP of cereal grains create viscous solutions in the gut as they aggregate into large network structures as a result of entanglements of many very large polymers (Morris and Ross-Murphy, 1981). To destroy such a network, it is not necessary to completely digest the polymers. The simple breakdown of the polymers into shorter pieces such that they no longer associate in such large entanglements is enough to destroy the viscosity. In order

to achieve that, a single enzyme should be effective in breaking down the backbone of the polymer.

However, in the experiment conducted by Pettersson and Aman (1988,1989), it was found that treatment of the rye-based diets with xylanase improved body weight gain of broiler chickens without any noticeable reduction in digesta viscosity. The authors concluded that although the pentosanase did destroy the gel-forming capacity of the soluble arabinoxylan fraction, it simultaneously led to the release of additional, and otherwise insoluble polymers from the cell wall, which maintained the overall high viscosity of the foregut contents. In this regard, a disruption of the intact walls and release of nutrients (i.e. protein, starch) was the major factor contributing to the improvement in the nutritive value ascribed to exogenous enzymes. In order to better disrupt the cell walls of the aleurone layer, Tervila-Wile et al.(1996) demonstrated in *in vitro* experiments that a combination of xylanase and cellulase was more effective in degrading the cell walls and solubilizing protein and carbohydrate components than when xylanase or cellulase were used alone (Table 8). In other experiments it was also reported that although β-glucanase alone was sufficient to disrupt barley endosperm walls, multi-enzyme preparations containing cellulase and xylanase maximized the release of protein from the aleurone layer (Murison et al., 1989; Mulder et al., 1991)

Basic differences in fibre components exist between monocotyledons and dicotyledons. Monocotyledons generally contain higher amounts of arabinoxylan and β-glucan and low levels of other polysaccharides, including pectic substances (Table 4). In contrast, dicotyledons contain high pectin levels, no pentosans and have xyloglucan as the major hemicellulase polymer. Based on the analyses, an enzyme supplement

containing pentosanases or glucanases should be selected for cereals while pectinases and cellulases would prove more useful for dicotyledon seeds such as soybean meal.

Protease enzymes have been used in animal feeds to target a wide range of feed ingredients. The exact mechanism by which they enhance the nutritional value is not known. Potential substrates could include some protein fractions poorly utilized by young animals or protein-containing anti-nutritional factors. In this context, protease inhibitors and lectins are widely distributed in oil-seeds. The soybean industry uses heat treatment to reduce or inactivate such factors. Laboratory studies have shown that some microbial proteases are capable of breaking down both protease inhibitiors and lectins (Classen et al. 1993; Meijer et al., 1993). In addition, protease, amylase and lipase supplements could be beneficial for young animals.

Today, a major pre-requisite to ensure the effectiveness of exogenous enzyme application would be to develop the "second-generation" enzyme cocktails which would target a variety of indigestible components of the diet and thus would contain many enzyme activities acting in concert to elicit a greater response than that from the single enzyme supplements. The challenge now is to design and to match the enzymes to the target substrates and specific components in the feeds in order to maximize the response from enzyme supplementation.

Table 8. Extractable protein and carbohydrate levels following in vitro digestion with xylanase and cellulase

Enzyme Used	Enzyme activity (U)	Crude protein (mg/g)	Total carbohydrate (mg/g)
None (control)	-	90[a]	60[a]
Xylanase	50	96[ab]	66[ab]
Xylanase +Cellulase	50+50	109[b]	77[b]

Source: Tervila-Wilo (1996)

3. MATERIALS AND METHODS

3.1. Materials

Commercial soybean meal and canola meal were obtained from Feed-Rite (Winnipeg, Manitoba) and a local crushing firm (Altona, Manitoba), respectively. Wheat was acquired from Purotone (Winnipeg, Manitoba). Hulless barley cv. Silky was obtained from the Glenlea Research Station, University of Manitoba. Enzyme supplements included xylanase, β-glucanase, α-amylase, amyloglucosidase, protease, cellulase A and cellulase B were provided by Canadian Bio-Systems, Calgary, Canada. Pepsin (P 7000), pancreatin (P 1750, 4 x USP), peptidase from porcine intestinal mucosa (P 7500) and pronase E (protease from *Streptomycers griseus*, Type XIV) were purchased from Sigma (St. Louis, MO, USA).

3.2. Isolation of wheat cell wall residues

Two preliminary in vitro experiments were conducted to select the best starch and protein hydrolyzing enzymes for an effective isolation of the cell wall residues of wheat.

An in vitro procedure developed in this laboratory (Simbaya et al, 1996; Slominski et al., 1999) was used in the study. In brief, a 5g sample was incubated with 50 ml of 0.1

M HCl/54 mM NaCl solution containing 250 mg of pepsin for 1 h at 40°C in an environmentally controlled incubator shaker (New Brunswick Scientific, Edison, USA). Following digestion with pepsin, the pH was adjusted to approximately 7.0 with 2.5 ml of 2.0 M NaOH. The pH was stabilized by adding 20ml of 0.1M phosphate buffer containing 0.05% sodium azide. The contents were then transferred into pre-soaked dialysis tubes (Spectrum, Houston, TX, USA) with a molecular weight cut off value of 12000 -14000. One ml of buffer solution containing 50 mg of pancreatin was then added and the tubes were closed allowing for a small air gap in the tube to facilitate continued mixing of the contents. To simulate the environment of the small intestine and to minimize the effect of end product inhibition, protein digestion with pancreatin was performed with the aid of the digestion/dialysis unit which was constructed by mounting a speed reducer motor onto a thermally controlled water bath filled with 0.05 M phosphate buffer pH 7.0. The motor was used to rotate a rectangular aluminium frame (32 x 18 cm) at a speed of 20 rpm. Attached to the metal frame were 6 dialysis tubes, each containing a sample of test material. Protein digestion with pancreatin was conducted for 6 h at 40°C. After incubation, enzyme activities were terminated by replacing the buffer with ice-cold distilled water and the contents were subjected to dialysis against ice-cold distilled water for 72 h with continuous rotation of the tubes. The water was changed regularly at 8 h intervals. Following dialysis, the contents were frozen, freeze-dried and analysed for protein and starch contents.

In vitro protein and starch digestibility was calculated by subtracting the amount of protein and starch remaining in the residue from that present in the original sample.

In the current study, an attempt has been made to further investigate the usefulness of the in vitro procedure for isolation of the cell wall residues from the high-starch products. Although many in vitro methods are based on consecutive incubation with pepsin and pancreatin, there is some evidence that the activity of starch-hydrolyzing enzymes in the pancreatin preparation could be a limiting factor in effective starch degradation (Babinszky et al. 1990; Boisen and Eggum 1991). Therefore, in the current study the wheat sample (ground to pass through a 1 mm sieve) was subjected to incubation with pepsin and pancreatin fortified with the starch hydrolyzing enzymes: α-amylase and amyloglucosidase. A boiled sample of wheat was also used in the study.

From the data presented in Table 9, it became evident that the wheat starch was not completely digested when subjected to incubate with pepsin-pancreatin (62.0%) or pancreatin fortified with α-amylase (61.0%). A significant increase in digestible starch content was observed for the boiled wheat sample (93.5%) indicating that the amount of starch hydrolyzing enzymes present in the pancreatin preparation is sufficient for an effective starch hydrolysis providing the starch is gelatinized prior to the assay. A similar increase in starch hydrolysis was observed for the "raw" wheat samples treated with varying amounts of amyloglucosidase. Therefore, amyloglucosidase was used further for isolation of the cell wall residue from the wheat samples.

Table 9. The effect of fortification of the pepsin/pancreatin system with starch hydrolyzing enzymes on in vitro digestible protein and starch contents of wheat (%).

Treatment	Digestible protein	Digestible starch
Control A (raw wheat[1]; pepsin + pancreatin)	82.7 ± 0.1[2]	62.0 ± 0.9
Control B (boiled wheat; pepsin + pancreatin)	84.1 ± 0.1	93.5 ± 0.4
Control A + α-amylase	81.6 ± 0.3	61.0 ± 1.4
Control A + amyloglucosidase	81.3 ± 0.8	83.1 ± 1.4
Control A + α-amylase + amyloglucosidase	80.5 ± 0.3	83.7 ± 0.9
Control A + α-amylase + amyloglucosidase (x2)	83.0 ± 0.1	91.5 ± 0.1

[1]Ground to pass through a 1mm sieve
[2]Mean ± SD

An additional study was conducted to determine any beneficial effect of fortification of the pancreatin preparation with the protein hydrolyzing enzymes. Two protein supplements, soybean meal and canola meal were used in this study. As shown in Table 10, incubation of canola meal or soybean meal with peptidase and bacterial protease (pronase) resulted in a varying degree of protein hydrolysis. As compared to the control treatment, addition of peptidase was found to be ineffective in improving the digestible protein content. however, pronase addition resulted in a significant increase in protein digestibility with the effect on soybean meal being more pronounced than that on canola. The positive effect of pronase addition agreed well with some earlier research in this laboratory (Simbaya et al., 1996) from which it was concluded that the positive effect of pronase addition was due to the presence of exo- and endopeptidases (Brillouet et al., 1988) in a manner similar to that of protein digestion in the small intestine with brush-border peptidases contributing to a more complete protein hydrolysis. However, it appeared evident from this study that this was not the case since the peptidase used in the current experiment was from porcine intestinal mucosa and did not show any improvement in protein digestibility. Therefore, pronase rather than peptidase was chosen for further in vitro studies.

Table 10: The effect of fortification of the pepsin/pancreatin system with protein hydrolyzing enzymes on in vitro digestible protein content of canola meal and soybean meal (%)

Treatment	Canola meal	Soybean meal
Control (pepsin + pancreatin)	75.4 ± 0.6[1]	74.8 ± 0.0
Control + peptidase	73.9 ± 0.1	76.7 ± 0.4
Control + pronase	81.7 ± 0.1	90.8 ± 0.7
Control + pronase + peptidase	80.3 ± 0.1	91.6 ± 0.0

[1] Mean ± SD

For the isolation of the cell wall residues, a commercial "as fed" wheat sample with the average diameter particle size of approximately 450 μm was used. Two different enzyme incubation procedures were employed for maximal nutrient release and cell wall material purification. Cell wall isolate A was prepared using the in vitro pepsin-pancreatin procedure described earlier (Simbaya et al. 1996; Slominski et al., 1999) with some modifications summarized in Fig. 5.

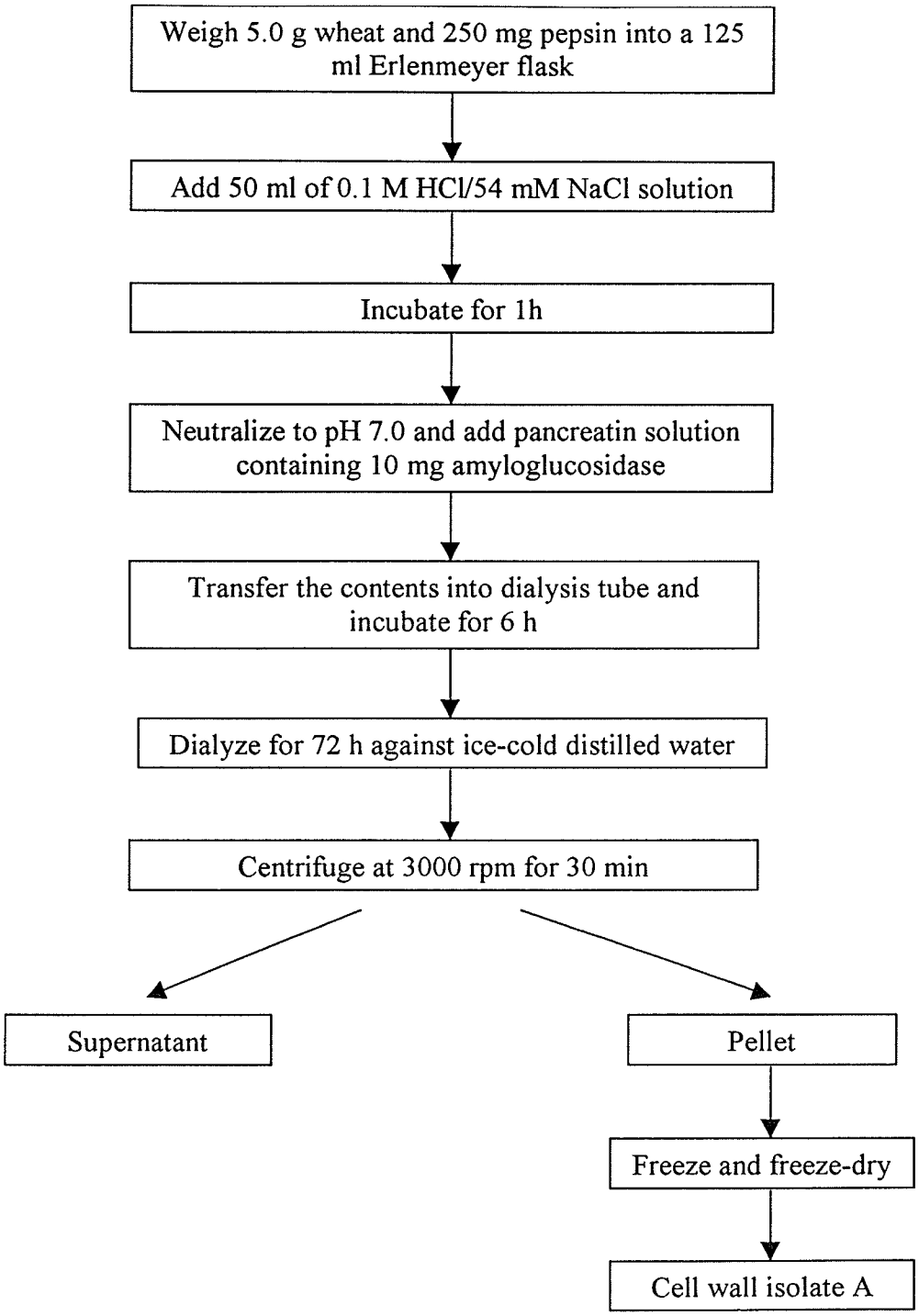

Figure 5. Procedure for isolation of the wheat cell wall residue A

Cell wall isolate B was obtained using a procedure outlined in Fig. 6 and described below.

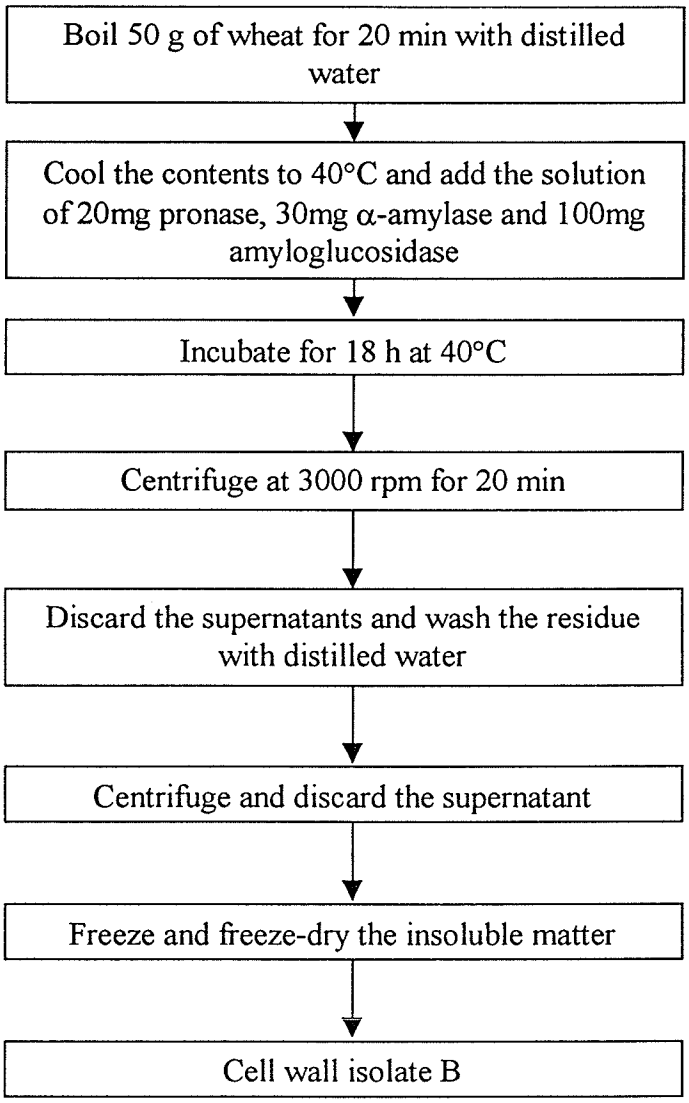

Figure 6. Procedure for isolation of the wheat cell wall residue B

Fifty grams of coarsely ground wheat was boiled with 400ml of distilled water in a boiling water bath for 20 min. The sample was then cooled to 40°C and following enzyme addition (20mg pronase, 30mg α-amylase,100mg amyloglucosidase) was incubated at 40°C for 18 h in a controlled-environment shaker with continuous mixing. Following centrifugation at 3000 rpm for 30 min, the insoluble fraction was washed with distilled water, centrifuged and the content (pellet) was collected and freeze-dried.

3.3. In vitro enzyme evaluation

The evaluation involved the incubation of the wheat isolates with a combination of commercial xylanase, β-glucanase and cellulase preparations in the presence of amylase and protease enzymes and the determination of the non-starch polysaccharides, protein and starch contents of the water-insoluble residues. In the assay, 2 g of the cell wall residue was incubated for 5 h, under continuous mixing, with a defined amount of enzyme in 30 ml of acetate buffer (0.1 M, pH 5.4) at 40°C. Following centrifugation at 3000 rpm for 20 min, the supernatant was discarded and the residue was washed with distilled water, centrifuged and the pellet freeze-dried. The freeze-dried residues were subjected to non-starch polysaccharide, protein and starch analyses and the effect of enzyme addition was determined by difference between non-enzyme and enzyme treatment.

A similar procedure was used to investigate the effect of enzyme addition on barley, canola meal and soybean meal cell wall polysaccharide depolymerisation and protein hydrolysis. In this procedure, 5 g of soybean meal, canola meal and coarsely ground

barley were incubated under continuous shaking with different combinations of carbohydrase enzymes in the presence or absence of protease in 30 ml of acetate buffer (0.1 M, pH 5.0) at 40°C. Following centrifugation at 3000 rpm for 30 min, the supernatant was discarded and the residues were washed with 50 ml distilled water, the contents were centrifuged again and the residue (pellet) was collected, freeze-dried and analyzed for protein, non-starch polysaccharide and phytate contents.

3.4. Enzyme compatibility study

It is well known that the bacterial and fungal enzymes recommended for animal feeds contain a variety of side activities. A common activity found in many preparations is a protease enzyme which, when used in combination with other enzymes, could inhibit their activity and affect the effectiveness of enzyme use. Therefore, the xylanase, β-glucanase and α-amylase preparations were tested for their activity under optimal temperature and pH conditions in the absence and presence of other commercial enzymes. Quest International standard analytical methods with some modifications (Slominski, University of Manitoba, 1998, unpublished) were used in this study. As shown in Table 11, the activities of xylanase and amylase were not affected by the addition of other enzyme preparations and even increased in some cases due to the xylanase and amylase side activities present in preparations under study. The glucanase activity, on the other hand, showed some decline in activity when used in combination with the protease preparation (please note that in this assay any difference in enzyme activity higher than 5% would be considered significant).

Table 11. Activity of various commercial enzymes when incubated alone or in combination with other preparations under optimal temperature and pH conditions.

Enzyme combination	Xylanase activity (units/g/min)
Xylanase	19,704
Xylanase + β-glucanase (1 : 1 w/w)	21,986
Xylanase + cellulase (2.5 : 1 w/w)	23,522
Xylanase + α-amylase (2.5 : 1 w/w)	21,598
Xylanase + amyloglucosidase (2.5 : 1 w/w)	24,415
Xylanase + protease (10 : 1 w/w)	22,341

Enzyme combination	β-Glucanase activity (units/g/min)
β-Glucanase	10,914
β-Glucanase + xylanase (1 : 1 w/w)	11,210
β-Glucanase + cellulase (2.5 : 1 w/w)	14,290
β-Glucanase + α-amylase (2.5 : 1 w/w)	12,213
β-Glucanase + amyloglucosidase (2.5 : 1 w/w)	12,439
β-Glucanase + protease (10 : 1 w/w)	9,918

Enzyme combination	α-Amylase activity (FAA units/g/min)
α-Amylase	979,847
α-Amylase + xylanase (1 : 4 w/w)	982,503
α-Amylase + amyloglucosidase (1 : 1 w/w)	1,255,447
α-Amylase + cellulase (1 : 1 w/w)	1,069,924
α-Amylase + protease (4 : 1 w/w)	980,230

3.5. In vivo enzyme evaluation

Two experiments were conducted to determine the effect of dietary enzyme supplementation on broiler chickens performance when fed wheat/hulless barley/soybean/canola meal-based diets.

One-day-old male Arbor Acres broiler chicks, vaccinated against Marek's disease, were purchased from a local commercial hatchery. During the first 4 days, the birds were housed in Jamesway brooder batteries and had free access to water and a commercial chick starter diet containing 20% protein. On Day 5, the birds were fasted for 4 hours before being individually weighed and placed into narrow weight classes. Groups of five birds were then assigned to pens in Petersime brooder batteries such that all pens had a similar initial weight. From Day 5 to Day 19, the birds were fed experimental diets that were in a mash form. The birds had free access to water and feed and were provided with continuous light. Pen weights were recorded on Day 12 and Day 19. Before each weighing, the birds were fasted for 4h. Feed consumption was recorded for week 1 and 2 in order to calculate weekly and overall feed intake and feed to gain ratio.

The basal diets used in both Experiment 1 and 2 were moderately deficient in protein, amino acids and metabolizable energy (Table 12). In formulation of experimental diets, the metabolizable energy contents assigned to wheat, barley, canola meal and soybean meal were 3140, 3150, 2000 and 2520 kcal/kg, respectively. Crude protein contents of commercial soybean meal, canola meal, wheat and hulless barley used in Experiment 1 were based on the determined values which averaged 44.0, 35.1, 13.0 and

13.3%, respectively. The crude protein contents of soybean meal, canola meal, wheat and hulless barley used in Experiment 2 was 46.5, 36.1, 12.4 and 16.4%, respectively

In Experiment 1, the chickens were randomly assigned to 3 dietary treatments (10 replicates/treatment) which included a control diet and two test diets supplemented with either enzyme blend A or B. Enzyme blend A contained conventional xylanase and glucanase while enzyme blend B consisted of xylanase, glucanase, amylase, protease, invertase and some cell wall degrading cellulases. In Experiment 2, a total of 4 enzyme supplemented diets were used. Composition of enzyme blends and enzyme activities and/or inclusion rates are shown in Tables 13 and 14.

Table 12. Composition and calculated analysis of basal diets used in Experiment 1 (Diet 1) and Experiment 2 (Diet 2)

	Diet	
	1	2
Ingredient (%)		
Canola meal	11.00	11.00
Soybean meal	18.90	18.00
Wheat	47.14	48.64
Hulless barley	15.70	15.00
Limestone[1]	1.60	1.70
Biophosphate[2]	1.25	1.06
Vitamin premix[3]	1.00	1.00
Mineral premix[4]	0.50	0.50
DL-methionine	0.08	0.07
Lysine	0.03	0.035
Alphacell	0.30	0.30
Vegetable oil	2.50	2.70
Calculated composition		
Metabolizable energy (kcal/kg)	2905	2906
Crude protein (%) (N×6.25)	21.00	21.00
Lysine (%)	1.00	1.00
Methionine (%)	0.46	0.46
Meth. + Cyst. (%)	0.84	0.85
Calcium (%)	1.00	1.00
Available phosphorus (%)	0.45	0.41

[1] Contained 380 g calcium per kilogram; [2] Contained 180 g calcium and 210 g phosphorus per kg; [3] Vitamin premix provided per kg of diet: vitamin A, 8250 IU; vitamin D_3, 1000IU; vitamin E, 11IU; thiamin, 0.012 mg; vitamin B_{12}, 0.012; vitamin K, 1.1 mg; niacin, 53 mg; choline, 1020 mg; folic acid, 0.75 mg; biotin, 0.25 mg, riboflavin, 5.5 mg; [4] 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

Table 13. Composition of enzyme blends used in broiler chicken experiments

	Experiment 1		Experiment 2			
	A	B	C	D	E	F
Xylanase	X	X	X	X	X	X
β-Glucanase	X	X	X	X	X	X
Invertase		X	X	X	X	X
α-Amylase[1]		X		X	X	X
Cellulase A		X			X	X
Cellulase B		X			X	X
Protease		X				X

[1] Includes α-amylase and amyloglucosidase

Table 14. Xylanase, glucanase, protease and amylase activities (units/kg diet) and cellulase content (%) of experimental diets

Treatment	Xylanase	β-Glucanase	Protease	Amylase	Cellulase
Experiment 1					
Control	-	-	-	-	-
Enzyme blend A	410	257	20	-	-
Enzyme blend B	470	390	1410	8094	0.001
Experiment 2					
Control	-	-	-	-	-
Enzyme blend C	410	257	27	-	-
Enzyme blend D	448	287	320	8094	-
Enzyme blend E	474	399	393	8094	0.0015
Enzyme blend F	441	380	1431	8405	0.0015

On Day 19 of each experiment, the excreta samples from each pen were collected, frozen, freeze-dried and pooled to obtain 4 samples per treatment. The samples were finely ground in a coffee grinder and were analyzed for chromic oxide (internal marker), gross energy (GE), nitrogen (Kjeldahl), NSP and phytate content. Apparent dry matter, NSP and phytate digestibilities and metabolizable energy (AME_n) contents were calculated as follows (Hill et al., 1960):

Diet digestibility (%) = $[1 - (Cr_2O_{3\ \%\ feed} / Cr_2O_{3\ \%\ excreta})] \times 100$

NSP digestibility (%) = $[1- (NSP_{\%\ excreta}/NSP_{\%\ feed})\times(Cr_2O_{3\%\ feed}/Cr_2O_{3\%\ excreta})]\times100$

Starch digestibility (%) = $[1- (Starch_{\%\ excreta}/Starch_{\%\ feed})\times(Cr_2O_{3\%\ feed}/Cr_2O_{3\%\ excreta})]\times100$

Phytate digestibility (%) = $[1- (phytate_{\%\ excreta}/phytate_{\%\ feed})\times(Cr_2O_{3\%\ feed}/Cr_2O_{3\ \%\ excreta})]\times 100$

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

At the end of the experiment, 8-10 birds per treatment were randomly selected for intestinal viscosity measurement. The bird was killed by cervical dislocation and the abdominal cavity was exposed. The contents of small intestine form the Meckel's diverticulum to 1.5 cm prior to the ileal-cecal junction were collected into two 2 ml microcentrifuge tubes and centrifuged at 9000 rpm for 5 minutes. Viscosity was

determined using the Brookfield digital viscometer (Model DV- II +, Brookfield Engineering Laboratories, Stoughton, MA.).

3.6. Chemical analyses

Feed and excreta samples were analyzed for gross energy using a Parr adiabatic oxygen bomb calorimeter. Crude protein (Kjeldahl N x 6.25) was determined using the AOAC (1990) method. Non-starch polysaccharides were determined by gas-liquid chromatography (component neutral sugars) and by colorimetry (uronic acids) using the procedures described by Englyst and Cummings (1984) with some modifications (Slominski and Campbell, 1990). Starch was determined using the NSP procedure in which starch gelatinization with dimethylsulfoxide was substituted by boiling the samples with water for 30 min. Starch hydrolyzing enzymes (i.e., α-amylase, pullulanase and amyloglucosidase) were excluded from the procedure and the starch content was calculated as total sample glucose (no enzyme added) minus NSP glucose. The content of phytate was determined according to a method of Hang and Lantzsch (1983). Chromic oxide was analyzed using the procedure developed by Williams et al.(1963).

3.7. Statistical analyses

Data were analyzed using the General Linear Models (GLM) procedure from SAS software (Statistical Analysis Systems Institute Inc., 1985) and treatment means separated using Student-Newman-Keuls (SNK) test.

4. RESULTS AND DISCUSSION

4.1. Isolation of the wheat cell wall material

As the objective of the in vitro enzyme evaluation in the enzyme developmental process was to document that the improvement in animal performance could be achieved not only by digesta viscosity reduction but also by opening the cells and releasing the nutrients encapsulated by the cell wall structure, two isolates of the cell wall material were prepared. The cell wall Isolate A was prepared utilizing the pepsin-pancreatin system which mimics the gastrointestinal conditions of the monogastric animal (Slominski et al., 1999). In the current study, however, the pancreatin preparation was fortified with the amyloglucosidase enzyme since the results of the preliminary trial (see Table 9 for details) indicated that this enzyme may be necessary for an effective starch hydrolysis. The sample of wheat used in this study represented a grain ground under commercial conditions typical for the feed industry. According to earlier data from this laboratory, the commercial "as fed" wheat sample would have the average particle size diameter of approximately 450 μm and could be considered a "coarse" wheat when compared to the sample ground to pass through a 1 mm sieve in the laboratory cyclotec sample mill. Therefore, the "as fed" or "coarse" sample of wheat was used for the isolation of the call wall material as it was assumed that any fine grinding would result in

an additional breaking of the cell structure and in this context would be irrelevant to the objective of this research.

Percent removal of protein, starch and dry matter on isolation of the wheat cell wall material is shown in Table 15. Although a substantial amount of protein was hydrolyzed and removed on preparation of the Isolate A, the starch remaining in the preparation was still significant. This is in contrast to the preliminary trial (see Table 9) in which starch hydrolysis was nearly complete (i.e., 91.5%) when finely ground sample of wheat and additional amyloglucosidase were used. Removal of protein on Isolate A preparation, on the other hand, indicated that less than 20% of protein could be encapsulated by the cell wall structure of the aleurone layer of the grain. Moreover, a significant amount of protein could be associated with the cell wall polysaccharides and represent a structural protein, which plays an important role in cell wall stability (Smits et al.,1996) This type of protein (i.e., glycoprotein) is for the most part insoluble in water and would also resist digestion. It is difficult to predict if the low removal of starch on Isolate A preparation was due to starch encapsulation since the large size of the cells within starchy endosperm would be considered susceptible to any physical forces on grinding. Therefore, a relatively low starch removal on Isolate A preparation could be a consequence of a slow hydrolysis rate of the native starch under conditions of the current experiment.

Table 15. Percent removal of protein, starch and dry matter on isolation of the wheat cell wall material using two different procedures.

Cell wall isolate	Protein	Starch	Dry matter
A	82.6 ± 0.8[1]	54.5 ± 0.8	53.7 ± 0.9
B	67.1 ± 0.1	90.6 ± 0.1	80.6 ± 1.4

[1] Mean ±SD

The procedure for Isolate B preparation was different in that it did not mimic the physiological conditions of the GI tract since boiling and treatment of the sample with bacterial enzymes for prolonged period of time were employed. Such conditions were in essence similar to those used in earlier studies on cell wall material isolation (Selvendran, 1984; Carre and Brillovet, 1986).

As shown in Table 15, 80.6 % dry matter was removed from the "coarse" wheat sample on preparation of the cell wall Isolate B. Although most of the starch (90.6%) was hydrolyzed after boiling of the sample and treatment with starch hydrolyzing enzyme, small quantities of starch remained unhydrolyzed. It is assumed that this fraction of starch represents the resistant starch (i.e., retrograded amylose) formed upon cooling of the sample (Bjorck et al., 1984). A distinct difference in starch hydrolysis between the two isolates was due to starch gelatinization, a process known to be essential for an effective and fast starch hydrolysis. It is uncertain, however, if the effective removal of starch on Isolate B preparation was due to the lack of starch encapsulation or was a consequence of cell disruption during swelling and gelatinization of the starch granules. As compared to the cell wall Isolate A, less protein (67 vs. 83%) was removed from the cell wall Isolate B indicating that the pepsin and pancreatin preparations were more effective in protein hydrolysis than pronase alone.

4.2. In vitro enzyme evaluation

Both cell wall Isolate A and B were used further for in vitro enzyme evaluation. The preparation were incubated with different combinations of exogenous enzymes to

investigate the effect of cell wall hydrolysis/depolymerization on the release of nutrients entrapped within the cells. Protease and α-amylase were used to further digest any potential intercellular protein or starch bodies in situations when the carbohydrase enzymes were effective in cell wall disruption. As shown in Table 16, a significant increase in non-starch polysaccharide (18.6%), protein (48.4%) and starch (51.3%) hydrolysis was observed following α-amylase and protease treatment. Further improvement in non-starch polysaccharide (NSP), protein and starch hydrolysis was evident when combinations of cell-wall-degrading enzymes xylanase and β-glucanase or xylanase, β-glucanase and cellulase were used. Since the aleurone layer and the endosperm cell walls of wheat contain predominantly arabinoxylans (85%) and some amounts of β-glucan and cellulose, more complete NSP hydrolysis in both "raw" and "boiled" isolates was observed following addition of xylanase, β-gluanase and cellulase (36%) than xylanase and β-glucanase (31%). In addition, cell wall degrading enzymes facilitated the hydrolysis of protein by protease enzyme (58% vs. 48%) suggesting that the intracellular protein bodies can be degraded and utilized by protease more effectively following hydrolysis of the cross-linked polysaccharides. This is in agreement with the results reported by Mulder et al. (1991) and Tervila-Wilo (1998) which demonstrated that the pretreatment of the aleurone cells with cell wall degrading enzymes increased the release of protein present in the cell wall residues.

There was no major effect of cell wall degrading enzymes on starch hydrolysis/solubilization when using the "raw" isolate A.

Table 16. Percent non-starch polysaccharide (NSP), protein and starch solubilization/ hydrolysis following incubation of the wheat cell wall Isolate A with exogenous enzymes.

Treatment/ Enzyme	NSP	Protein	Starch
As is isolate (control)	-2.8 ± 0.4[d]	23.1 ± 3.4[d]	12.6 ± 0.4[e]
Enzyme A (α-amylase[1] + protease[2])	18.6 ± 1.0[c]	48.4 ± 0.2[c]	51.3 ± 0.4[d]
Enzyme A + xylanase[3] + β-glucanase[4]	31.5 ± 0.8[b]	56.5 ± 0.9[a]	51.9 ± 0.3[d]
Enzyme A + xylanase + β-glucanase +cellulase[5]	36.4 ± 3.1[a]	58.2 ±0.7[a]	53.2 ± 0.4[c]
Boiled isolate			
Enzyme A (α-amylase + protease)	27.9 ± 0.8[b]	46.3 ±0.7[c]	98.5 ± 0.2[b]
Enzyme A + xylanase + β-glucanase	31.5 ± 2.0[b]	50.9 ±0.6[b]	99.3 ± 0.3[a]
Enzyme A + xylanase + β-glucanase +cellulase	37.7 ± 1.9[a]	55.7 ±0.3[a]	99.4 ± 0.2[a]

[1] 124 units/g isolate; [2] 204 units/g isolate; [3] 100 units/g isolate; [4] 50 units/g isolate; [5] 50 units/g isolate; [6] Mean ±SD; [abcd] Values within columns with no common superscripts differ significantly (P≤0.05)

Similar improvement in protein and starch digestibility was observed for the boiled cell wall Isolate A treated with the same cell wall degrading enzymes. However, following boiling the hydrolysis of starch was nearly complete which is in agreement with many reports demonstrating that heat treatment and starch gelatinization have a positive effect on starch digestibility and NSP solubilization (Booher et al., 1951; Bjorck et al., 1989).

A complete starch hyrolysis in the boiled isolate A indicates that there is no physical barrier associated with the cell wall structure which could prevent any access of the enzyme to the substrate. As indicated earlier, however, any potential cell disruption by gelatinized starch can not be excluded.

The exogenous enzyme combinations were evaluated further using the cell wall Isolate B. Since starch was almost completely removed on Isolate B preparation, α-amylase was excluded from this experiment. As shown in Table 17, a similar trend towards increased NSP solubilization/hydrolysis was observed with a combination of xylanase, glucanase and cellulase enzymes. In contrast to the experiment with Isolate A, no effect of enzyme treatment on protein hydrolysis was observed.

Table 17. Percent protein and non-starch polysaccharide (NSP) solubilization/hydrolysis following incubation of the wheat cell wall Isolate B with exogenous enzymes

Treatment / Enzyme	Protein	NSP
Control (as is isolate; no enzyme)	14.6 ± 0.3^{5b}	5.7 ± 2.3^{d}
Protease[1]	27.8 ± 0.9^{a}	6.4 ± 2.2^{d}
Protease + xylanase[2]	29.3 ± 1.3^{a}	14.1 ± 0.7^{c}
Protease + xylanase + β-glucanase[3]	30.4 ± 0.4^{a}	16.9 ± 0.9^{b}
Protease + xylanase + β-glucanase + cellulase[4]	29.9 ± 0.9^{a}	20.0 ± 0.3^{a}

[1] 204 units/g isolate; [2] 100 units/g isolate; [3] 50 units/g isolate; [4] 50 units/g isolate; [5] Mean ± SD; [abc] Values within columns with no common superscripts differ significantly

Further to research on the cell wall isolates, a sample of "coarse" barley grain was subjected to incubate with different enzyme combinations of commercial protease, β-glucanase and cellulase preparations (Table 18). The cellulase preparation was claimed by the manufacturer to contain cellulase as a main activity and some other enzyme side activities including β-glucanase, xylanase and protease. The use of protease had a significant effect on both protein and NSP solubilization/hydrolysis. Addition of glucanase resulted in slightly increased cell wall polysaccharide hydrolysis while β-glucanase in concert with cellulase showed the highest degree of NSP depolymerization (67%). Contrary to the Isolate A, only a slight increase in protein digestibility (67% vs. 69%) was observed for the combination of the cell wall degrading enzymes. It was probably due to some of the wall inserted proteins linked to the 1,3;1,4-β-glucan components. Association of β-glucan with protein and different molecular size of β-glucan –protein complex may both affect protein solubility.

Table18. Percent protein and non-starch polysaccharide (NSP) solubilization/hydrolysis following incubation of "coarse" barley with exogenous enzymes

Treatment / Enzyme	Protein	NSP
Control (no enzyme)	17.6 ± 0.3^{b}	39.9 ± 0.0^{4c}
Protease[1]	67.7 ± 0.8^{a}	53.7 ± 0.1^{b}
Protease + β-glucanase[2]	69.5 ± 0.2^{a}	57.7 ± 1.4^{b}
Protease + cellulase[3]	69.6 ± 1.0^{a}	67.4 ± 0.2^{a}
Protease + β-glucanase + cellulase	69.5 ± 0.6^{a}	67.6 ± 1.2^{a}

[1] 204 units/g; [2] 50.4 units β-glucanase/g; [3] 3.3 mg cellulase/g; [4] Mean ± SD
[abc] Values within columns with no common superscripts differ significantly ($P \leq 0.05$)

The results of two commercial cellulase enzymes evaluation in the absence and presence of protease using soybean meal as a substrate are shown in Table 19. Both cellulase A and B were claimed by the manufacturer to contain several carbohydrase type activities in addition to the cellulase activity. In the absence of the protease activity, both enzymes showed no effect on NSP solubilization. There was a slight increase in protein solubilization following cellulase B addition, which probably resulted from the protease side activity present in this particular cellulase preparation. The phytate content of the non enzyme treated control sample demonstrated that approximately 50% of the total phytate present in soybean meal would appear to be water-soluble. In addition, a slight increase in water-soluble phytate content following incubation with the cellulase preparation was again a result of some protease activity present in the cellulase preparation. This was substantiated by a significant increase in soluble phytate content following incubation of soybean meal with the protease preparation (57.1 vs. 46.8%). However, no further improvement in phytate solubilization was noted for protease combined with either cellulase A or B. This would indicate that the remaining portion of soybean phytate (i.e., 42%) is associated with other than protein components. There was no effect of cellulase enzymes on dry matter, protein and NSP solubilization when used in combination with the protease preparation.

Table 19. Percent dry matter, protein, non-starch polysaccharide (NSP) and phytate solubilization/hydrolysis following incubation of soybean meal with exogenous enzymes.

Treatment / Enzyme	Dry Matter	Protein	NSP	Phytate
Control (no enzyme)	29.4 ±2.4[4b]	11.8 ± 0.2[c]	13.0 ± 1.2[a]	46.8 ± 0.1[d]
Cellulase A[1]	30.0 ± 2.8[b]	11.5 ± 0.3[c]	8.9 ± 0.5[a]	46.4 ± 0.1[d]
Cellulase B[2]	31.8 ± 3.2[b]	13.1 ± 0.3[b]	9.5 ± 1.8[a]	49.8 ± 0.4[c]
Cellulase A + cellulase B	31.4 ± 2.7[b]	15.7 ± 1.0[b]	7.4 ± 0.4[a]	47.8 ± 0.4[b]
Protease[3]	44.9 ± 3.1[a]	40.1 ± 0.1[a]	10.3 ± 0.5[a]	57.1 ± 0.4[a]
Protease + cellulase A	45.0 ± 2.3[a]	40.2 ± 0.1[a]	10.3 ± 0.2[a]	57.7 ± 0.3[a]
Protease + cellulase B	45.1 ± 1.3[a]	40.3 ± 0.3[a]	13.6 ± 4.4[a]	57.9 ± 0.8[a]
Protease + cellulase A + cellulase B	45.4 ± 0.2[a]	40.5 ± 0.5[a]	14.6 ± 5.8[a]	57.5 ± 0.1[a]

[1] 2 mg/g meal; [2] 2 mg/g meal; [3] 100 units/g meal; [4] Mean ± SD

[abc] Values within columns with no common superscripts differ significantly (P≤0.05)

In contrast to soybean meal, both cellulase enzymes significantly improved hydrolysis/solubilization of canola meal NSP (Table 20). It is not clear, however, why cellulase B alone showed higher activity towards NSP solubilization (20.9%) when used alone than when used in combination with cellulase A (10.6%) or protease (11.7%). As expected, addition of protease significantly improved protein hydrolysis. However, there was no further improvement in protein solubilization following cellulase A or B addition. Similarly to the experiment with soybean meal, a slight increase in protein solubilization following cellulase B addition was a consequence of some protease side activity present in this preparation. Contrary to the experiment with soybean meal, there was no effect of either enzyme, including the protease preparation, on phytate solubility. In addition, the solubility of phytate was found to be very low in canola meal which may have some repercussions in relation to the effective use of phytase enzyme in canola meal-based diets.

Table 20. Percent dry matter, protein, non-starch polysaccharide (NSP) and phytate solubilization/hydrolysis following incubation of canola meal with exogenous enzymes.

Treatment / Enzyme	Dry Matter	Protein	NSP	Phytate
Control (no enzyme)	25.0 ±0.1[4a]	18.8 ±0.3[c]	7.0 ± 0.1[e]	6.1 ± 2.4[a]
Cellulase A[1]	25.5 ±0.7[a]	17.8 ±0.1[c]	10.7 ± 1.4[d]	4.0 ± 1.0[a]
Cellulase B[2]	26.0 ±0.9[a]	21.3 ±0.2[b]	20.9 ± 0.9[a]	14.7 ± 2.8[a]
Cellulase A + Cellulase B	26.8 ±1.0[a]	21.3 ±0.1[b]	10.6 ± 0.6[d]	9.5 ± 0.1[a]
Protease[3]	27.8 ± 0.1[a]	31.8 ± 0.3[a]	7.9 ± 0.2[e]	1.8 ± 5.9[a]
Protease + Cellulase A	29.5 ± 0.3[a]	31.5 ± 0.4[a]	14.2 ± 1.0[c]	5.2 ± 2.4[a]
Protease + Cellulase B	29.0 ± 0.1[a]	31.5 ± 1.6[a]	11.7 ± 0.6[d]	6.7 ± 0.2[a]
Protease + Cellulase A + Cellulase B	31.1 ± 0.6[a]	32.2 ± 0.0[a]	18.0 ± 0.5[b]	10.1 ± 5.8[a]

[1] 2 mg/g meal; [2] 2 mg/g meal; [3] 100 units/g meal; [4] Mean ±SD

[abc] Values within columns with no common superscripts differ significantly (P≤0.05)

4.3. In vivo enzyme evaluation

4.3.1. Animal Experiment 1

The results of a 2-week performance of broiler chickens fed wheat/hulless barley/soybean/canola meal-based diet supplemented with two different blends of enzymes are given in Table 21. There was no effect of enzyme supplementation on feed consumption. A diet supplemented with Enzyme A, which contained mainly the xylanase and β-glucanase activities, slightly increased body weight gain but, significantly improved feed conversion ratio (1.54 vs.1.58). Enzyme blend B composed of α-amylase, amyloglucosidase, protease and cellulase in addition to xylanase and β-glucanase preparations showed a trend toward further improvement in body weight gain and feed to gain ratio then.

Table 21. Effect of enzyme supplementation on broiler chicken performance.

Treatment	Feed Intake (g/bird/14days)	Weight Gain (g/bird/14days)	Feed : Gain Ratio
Control (no enzyme)	692 ± 33.3[a1]	440 ± 25.4[a]	1.58 ± 0.04[a]
Enzyme A	688 ± 13.4[a]	447 ± 9.5[a]	1.54 ± 0.03[b]
Enzyme B	695 ± 33.8[a]	460 ± 23.2[a]	1.51 ± 0.03[b]

[1] Mean ± SD; [a,b,c] Values within columns with no common superscripts differ significantly ($P \leq 0.05$)

When compared to control treatment, both Enzymes A and B significantly ($P \leq 0.05$) reduced intestinal viscosity and no difference in digesta viscosity between the two enzyme supplements was observed (Table 22). Therefore, Better response from Enzyme B supplementation on chicken performance was probably related to the augmentation of the animal's own secretary capacity by starch- and protein-hydrolyzing enzymes present in Enzyme B and/or the reduction of nutrient encapsulating effect of the cell walls. Nitsan et al. (1991), Noy and Sklan (1995) and Jin et al. (1998) reported that the specific activity of amylase, trypsin and lipase in broiler chickens rapidly increased up to 2-3 weeks of age after which time the activities plateaued and remained constant. It has been emphasized that the immaturity of the digestive system in young poultry may result in poor utilization of dietary nutrients (Jin et al., 1998). In addition, it is believed that the use of an effective combination of cell wall-degrading enzymes would reduce the nutrient encapsulating effect of the cell walls and could result in an increase in protein and starch utilization (Mulder et al. 1991; Murison et al., 1989).

The improvement in broiler chicken performance with enzyme supplementation was further reflected in the AME_n contents (Table 22). As compared to the control treatment, Enzyme A and B significantly increased the contents of dietary AME_n by 6.4 and 11.6%, respectively. Similar improvement with enzyme supplementation was observed for starch, phytate and NSP digestibilities (Table 23). Enzyme A and B improved starch digestibility by 3.9 and 7.0%, respectively. Since starch is the major dietary energy source, improvement in starch digestibility following enzyme supplementation was the main contributor to the increase in AME_n value of the enzyme supplemented diets. With regard to the NSP digestibility, broiler chickens fed a control diet were able do digest

only 3.7% of the total NSP. The digestibility value was close to the apparent digestibility of total NSP found by Slominski (1992), but was lower than that reported by Petterson and Aman (1989). Addition of enzyme significantly improved NSP digestibility (Table 23). It was evident that Enzyme B was more effective in improving NSP digestibility, presumably due to the presence of more diversified combination of cell-wall degrading enzymes in this preparation. It is believed that the improvement in NSP digestibility contributed, at least to some extent, to the overall energy utilization. In this context, the non-starch polysaccharides have been shown to be inversely related to energy digestibility (Steenfeldt and Jensen, 1998a,b) and the metabolizable energy content of feedstuffs could be improved by enzyme supplementation that renders the NSP more available for hindgut fermentation. The main end-products of microbial fermentation are short chain fatty acids which provide the host with energy. Although in poultry, the contribution of energy derived from the NSP fermentation would be small, the improvement in energy utilization could be of much higher magnitude if an appropriate enzyme supplement was used.

Table 22. Effect of enzyme supplementation on digesta viscosity and AME_n content

Treatment	Viscosity (cps)	AME_n (kcal/kg)
Control	4.71 ± 1.0[1a]	2515.3 ± 25.2[c]
Enzyme A	2.80 ± 0.6[b]	2676.3 ± 15.1[b]
Enzyme B	2.90 ± 0.6[b]	2809.8 ± 9.1[a]

[1] Mean ± SD; [a,b.c] Values within columns with no common superscripts differ significantly ($P \leq 0.05$)

The digestibility of phytate in broiler chickens fed a control diet was found to average 26% (Table 23) indicating that the young broiler chicken is to some extent capable of hydrolyzing the dietary phytate. This is in contrast with some studies showing phytate phosphorus to be largely unavailable to the birds as they lack the appropriate enzyme activity. However, some recent data (Maenz et al., 1997) indicated that the small intestinal brush border membrane of chickens in fact contains some phytase activity with the highest activity present in the duodenum. In addition, considerable levels of phytase activity are present in cereal grains such as wheat (i.e., 1193 units/kg) or barley (ie., 582 units/kg) (Ravindran et al., 1995). Therefore, both endogenous phytase activities may contribute to the partial digestibility of dietary phytate. In addition, it appeared evident that Enzyme B further improved phytate digestibility (33.2%; $P<0.05$) which could be related to the protease and some phytase side activities present in this preparation. It is well known that the phytate occurs as crystals associated with the protein bodies principally in the aleurone layer of the grain (Selvendran et al., 1987). It is therefore assumed that the improvement in phytate digestibility could be associated with the hydrolysis of the phytate-protein complex by a non-specific protease. This could then result in improved phytate dephosphorylation by either the endogenous phytase of the small intestine or phytase present in the diet.

Table 23. Effect of enzyme supplementation on digestibility of selected dietary components in broiler chicken (%)

Treatment	Dry matter	Starch	NSP	Phytate
Control	61.0 ± 1.1^{1c}	91.7 ± 2.8^{c}	3.7 ± 1.7^{c}	26.0 ± 5.3^{b}
Enzyme A	64.7 ± 0.9^{b}	95.2 ± 0.4^{b}	12.7 ± 1.9^{b}	26.6 ± 3.2^{b}
Enzyme B	67.8 ± 1.9^{a}	98.1 ± 0.6^{a}	20.7 ± 2.8^{a}	33.2 ± 2.1^{a}

[1] Mean ± SD; [a,b.c] Values within columns with no common superscripts differ significantly ($P \leq 0.05$)

4.3.2. Animal Experiment 2

The results of Experiment 1 showed a significant response from enzyme supplementation. Therefore, a second study (Experiment 2) was carried out using different combinations of exogenous enzymes to identify the preparations with the highest effect on animal performance and nutrient digestibility. As shown in Table 12, a similar basal diet was used in this study. Enzyme blends evaluated included Enzyme C (xylanase, β-glucanase and invertase), Enzyme D (xylanase, β-glucanase, invertase, amylase and amyloglucosidase) and Enzyme E (xylanase, β-glucanase, invertase, amylase, amyloglucosidase and two different preparations of cellulase. Enzyme F was similar to that of Enzyme B used in Experiment 1 and contained xylanase, glucanase, invertase, amylase, amyloglucosidase, cellulase A and B, and protease (Tables 13 and 14).

Because the results for week 1 and 2 showed similar trends, only the overall data are presented (Table 24). When compared to the control treatment, enzyme supplementations tended to improve broiler chicken performance. Among the four enzyme treatments, Enzyme F showed the most pronounced response. When compared to control, feed consumption, weight gain and feed to gain ration improved by 5.7, 6.0 and 3.2%, respectively. These results were similar to those of Experiment 1, except that the average body weight gain of chickens was lower. As shown in Table 25, the improvement in chicken performance with enzyme supplementation was partially due to the viscosity reduction. Although the four enzyme-supplements reduced the viscosity, the intestinal viscosity of chickens fed a control diet was much higher than that observed in Experiment 1. In addition, although the viscosity reduction due to enzyme

supplementation was significant, it did not reach the low level (2.8 cps) observed in experiment 1.

Enzyme supplementation improved ($P<0.05$) AMEn content significantly (Table 25), with the improvement ranging from 1.8 to 4.5%. The highest improvement in AME content (i.e., 3024.5 kcal/kg) was noted for Enzyme F. A significant improvement in AME_n content for Enzyme F supplemented diet was probably due to further fortification of this particular blend with the protease enzyme. Therefore, an increase in energy availability was a result of improved protein utilization in a young animal system. It is uncertain why the AME_n content of a basal diet used in this experiment (i.e., 2893 kcal/kg) was higher than that observed earlier in Experiment 1 (i.e., 2515 kcal/kg). Consequently, the improvement in AME_n content (i.e., 130 kcal/kg) following enzyme supplementation in Experiment 2 was less pronounced than that seen in Experiment 1 (ie., 294 kcal/kg). Variability in the nutritive content of dietary ingredients could explain this difference. In this regard, the hulless barley used in Experiment 2 was trashed twice and was found to contain significantly less fibre and more protein (16.7%) when compared to the protein content (13.3%) of hulless barley used in Experiment 1.

The digestibility of total NSP, starch and phytate is given in Table 26. The improvement in NSP digestibility was statistically significant ($P<0.05$) only when Enzyme F was used. This would indicate that only a combination of various carbohydrases and a nonspecific protease was effective in cell wall disruption. This is in agreement with some earlier studies indicating that the dietary fibre fraction can be effectively hydrolyzed and utilized only when a multi-enzyme preparation is used (Mulder et al. 1991; Murison et al. 1989). Interestingly, the digestibility of NSP in the

control treatment approached 14% and was much higher than that observed in Experiment 1 and in some earlier studies from this laboratory (Slominski, 1992). Higher viscosity and NSP digestibility observed in the control treatment indicated that the feed ingredients used in Experiment 2 (ie., hulless barley) contained much higher level of water-soluble NSP. In this context, soluble NSP are known to influence the intestinal viscosity and be fermented by the microflora of the lower gut to much greater extent than their insoluble counterparts (Smiths and Annison, 1996).

Starch digestibility of the control treatment, on the other hand, was only slightly higher (i.e., 93.8%) in Experiment 2 than in Experiment 1 (i.e., 91.7%). Although the addition of enzyme increased starch digestibility, there was no difference in starch digestibility among the four enzyme-supplemented diets. It is unclear why starch digestibility increased significantly following Enzyme C supplementation and remained constant when Enzyme D containing starch-hydrolyzing enzymes was used. This contradicts with the hypothesis that the lower secretion of endogenous amylase by young broiler chickens is responsible for incomplete starch digestibility.

When compared to the control treatment, phytate digestibility increased by 24.5 % when Enzyme F was used. There was no effect of Enzyme C, D and E on phytate digestibility. It would appear evident from this study that further fortification of the enzyme blend with protease (i.e., Enzyme F) not only improved energy utilization but also improved phytate P availablity.

Table 24. Effect of enzyme supplementation on broiler chicken performance

Treatment	Feed Intake (g/bird/14days)	Weight Gain (g/bird/14days)	Feed :Gain Ratio
Control (no enzyme)	598 ± 28.4^{1b}	385 ± 18.5^{a}	1.56 ± 0.03^{a}
Enzyme C	605 ± 9.9^{ab}	392 ± 9.1^{a}	1.54 ± 0.03^{a}
Enzyme D	610 ± 16.4^{ab}	400 ± 14.6^{a}	1.53 ± 0.03^{a}
Enzyme E	587 ± 30.7^{b}	383 ± 23.7^{a}	1.53 ± 0.04^{a}
Enzyme F	632 ± 21.3^{a}	408 ± 12.7^{a}	1.51 ± 0.03^{a}

[1] Mean ± SD; [a,b.c] Values within columns with no common superscripts differ significantly ($P \leq 0.05$)

Table 25. Effect of enzyme supplementation on intestinal viscosity and AME_n content

Treatment	Viscosity (CPs)	AME_n (kcal/kg)
Control (no enzyme)	11.1 ± 5.3^{1a}	2893.8 ± 6.0^{c}
Enzyme C	4.8 ± 1.3^{b}	2968.5 ± 26.5^{b}
Enzyme D	6.2 ± 2.6^{ab}	2946.4 ± 19.8^{b}
Enzyme E	3.8 ± 0.8^{b}	2959.7 ± 15.7^{b}
Enzyme F	5.5 ± 1.8^{b}	3024.5 ± 22.9^{a}

[1] Mean ± SD; [a,b.c] Values within columns with no common superscripts differ significantly ($P \leq 0.05$)

Table 26. Effect of enzyme supplementation on digestibility of selected dietary components in broiler chicken (%)

Treatment	Dry matter	Starch	NSP	Phytate
Control (no enzyme)	66.5 + 3.0[a1]	93.8 ± 2.5[b]	3.7 ± 4.9[b]	27.1 ± 1.3[b]
Enzyme C	68.3 ± 0.6[a]	98.4 ± 0.3[a]	16.9 ± 4.0[ab]	30.8 ± 4.2[ab]
Enzyme D	68.4 ± 2.8[a]	98.7 ± 0.7[a]	13.9 ± 2.2[b]	27.0 ± 2.6[b]
Enzyme E	68.5 ± 2.9[a]	98.5 ± 0.7[a]	19.0 ± 3.3[ab]	32.8 ± 4.8[ab]
Enzyme F	69.8 ± 1.2[a]	98.4 ± 0.4[a]	24.5 ± 1.4[a]	33.7 ± 0.5[a]

[1] Mean ± SD; [a,b.c] Values within columns with no common superscripts differ significantly (P≤0.05)

In these two experiments, apparently all enzyme preparations contained, to a certain extent, xylanase and β-glucanase in addition to other enzyme activities. Overall, the results showed improved apparent nutrient digestibilities in the groups given the enzyme-supplemented diets and, as the consequence, increased bird performance.

Improvement in performance of broiler chickens fed diets supplemented with enzymes observed in the current study is in agreement with the results reported earlier (Petterson et al., 1990; Bedford and Classen, 1992; Friesen et al., 1992; Marquardt et al., 1994; Veldman and Vahl, 1994; Choct et al., 1995). The positive effect of enzyme supplementation on growth performance observed in the current study could be explained, partially, by a decrease in viscosity of the intestinal contents, presumably due to the breakdown of arabinoxylan and β-glucan.

The results of the present investigation also showed higher apparent nutrient digestibilities and AMEn in the groups offered the enzyme-supplemented diets. The best response was obtained from diets supplemented with a multi-enzyme preparation containing cell-wall-degrading enzymes, nonspecific protease as well as starch-hydrolyzing enzymes (ie., amylase, amyloglucosidase). It was shown that a substantial amount of protein is associated with the cell wall fraction and, as indicated by Bacic and Stone (1986), this portion of protein appears to be covalently bound to the cell wall polysaccharides which are very stable during processing and cannot easily be digested by monogastric animals. In the research conducted by Bach Knudsen et al. (1995), whole wheat kernels and different fractions of the wheat were pretreated with the enzyme preparations containing xylanase, β-glucanase, cellulase and other enzyme activities. The pretreatment resulted in a significant depolymerisation and solubilisation of the cell wall

polysaccharides (NSP) and starch. This is in agreement with the results of our in vitro experiments, indicating that pretreatment of the cell wall residue with a combination of cell wall degrading enzymes may disrupt the cell wall structure and disclose the valuable nutrients. A similar trend was observed by Choct et al. (1995) who found that supplementation with the NSP-degrading enzymes significantly increased the NSP solubilisation in the small intestine of broiler chickens fed diets containing high levels of wheat (80%). In another study, the improvement in NSP digestibility was reported to contribute more available energy to the chicken (Jorgensen et al., 1996). In a study by Annison (1991), an increase of 8-11% in ileal starch digestibility and AME content was reported for enzyme-supplemented diets. All these results, including the data presented above, indicate that the effect of enzyme supplementation is a function of nutrients released following disruption of the polysaccharides-rich aleurone and endosperm cell walls and of reduced digesta viscosity.

It is concluded from the current study that the improvement in broiler chicken performance with enzyme supplementation was a consequence of increased nutrient digestibility and AMEn value. Some positive effects were also related to the reduced viscosity of digesta. It was documented that the enzymes are capable of degrading the cell wall fibre since the overall improvement to the nutritive value of experimental diets was accompanied by an increase in apparent digestibility of total NSP.

5. CONCLUSIONS

1. A substantial amount of protein and polysaccharides is present in the wheat cell wall residue and is, for the most part, water-insoluble and resistant to digestion.
2. A multi-cell-wall-degrading-enzymes preparation had more effect on wheat and barley NSP solubilization than s single enzyme preparation. However, the release of protein present in the cell wall residues was not consistent throughout the study.
3. Carbohydrase preparations evaluated in the current study showed no effect on soybean meal NSP solubilization, but improved hydrolysis/solubilization of canola meal NSP.
4. Enzyme addition resulted in improved nutrient digestibility and broiler chicken performance.
5. It is hypothesized that the effectiveness of enzyme addition was highly dependent on the reduction of digesta viscosity, augmentation of the animal's own digestive enzymes and disruption of the intact walls and the release of entrapped nutrients.
6. Multi-enzyme preparations were more effective in hydrolyzing a variety of substrates and specific components in the feeds than the conventional xylanase and glucanase preparation.

6. REFERENCES

ALLEN,G.M., BEDFORD, M.R. and McCRACKEN, K.J. 1996. Effect of wheat inclusion and enzyme supplementation on diet metabolisability and broiler performance. *British poultry Science 37: s44-s45.*

ANNISON, G. 1991. Relationship between the levels of soluble nonstarch polysaccharides and the apparent metabolizable energy of wheats assayed in broiler chickens. *Journal of Agriculture and Food Chemistry 39: 1252-1256*

ANNISON, G. and CHOCT, M. 1991. Anti-nutritive activities of cereal non-starch polysaccharides in broiler diets and strategies minimizing their effects. *World Poultry Science Journal, Vol.47, November 1991*

ANNISON, G. 1992. Commercial enzyme supplementation of wheat–based diets raises ileal glycanase activities and improve AME, starch and pentosan digestibility in broiler chickens. *Animal Feed Science and Technology 38:105-121*

ANTONIOU, T. and MARQUARDT, R.R. 1982. Utilization of rye diets by chicks as affected by lipid type and level and penicillin supplementation. *Poultry science 61: 107-116*

ANTONIOU, T. and MARQUARDT, R.R. 1981. Influence of rye pentosans on the growth of chicks. *Poultry Science 60:1898-1904*

ANTONIOU, T., MARQUARDT, R.R. and CANSFIELD, E. 1981. Isolation, partial characterization and antinutritional activity of a factor (pentosans) in rye grain. *Journal of Agriculture and Food chemistry 29:1240-1247*

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (AOAC), 1990. Official methods of analysis. Helrich, K. (editor). AOAC Inc. Arlington, VA, USA.

BACH KNUDSEN, K.E., STEENFELDT,S., BORSTING, C.F., EGGUM, B.O. 1995. The nutritive value of decorticated mill fractions of wheat. 1. Chemical composition of raw and enzyme treated fractions and balance experiments with rats. *Animal Feed Science and Technology 52: 205-225*

BACIC, A. and STONE, B.A. 1981. Isolation and ultrastructure of aleurone cell walls from wheat and barley. *Australian Journal of Plant Physiology, 8: 453-474*

BAIDOO, S.K., SHIVES, A, and ROBBLEE, A.R. 1991. Effect of kernel density on the apparent and true metabolizable energy value of corn of chicken. *Journal of Poultry Science 70: 2102-2107*

BEDFORD, M.R. 1993. Mode of action of feed enzymes. *Journal of Apply Poultry Research 2:85-92*

BEDFORD, M.R. 1995. Mechanism of action and potential environmental benefits from the use of feed enzymes. *Animal Feed Science and Technology 53: 145-155*

BEDFORD, M.R. and CLASSEN, H.L. 1992b. Reduction of intestinal viscosity on barley-fed broilers by β-glucanase: site of action and effect on bird performance. *Animal Production : 54-88.*

BEDFORD, M.R. and CLASSEN, H.L. 1992. Deduction of intestinal viscosity through manipulation of dietary rye and pentosanase concentration is effected through

changes in the carbohydrate composition of the intestinal aqueous phase and results in improved growth rate and feed conversion efficiency of broiler chicks. *Journal Nutrition 122: 560-569.*

BEDFORD, M.R. and MORGAN, A.J. 1996. The use of enzymes in poultry diets. *World's Poultry Science Journal, Vol.52, March: 60-68*

BEDFORD, M.R.; CLASSEN, H.L. and CAMPBELL, G.L. 1991. The effect of pelleting, salt and pentosanase on the viscosity of intestinal contents and the performance of broilers fed rye. *Poultry Science 70: 1571-1577.*

BEDFORD,M.R. 1997. Factors affecting response of wheat based diets to enzyme supplementation. *In Recent advances in animal nutrition in Australia. Pp.1-7*

BELL, J.M. 1993. Factors affecting the nutritional value of canola meal: A review Canadian *Journal of Animal Science 73: 679-697*

BJORCK, I., GUNNARSSON, A., OSTERGARD, K. 1989. A study of native and chemistry modified potato starch. Part II. Digestibility in the rat intestinal tract. *Starke 41:128-134.*

BOOHER,L.E., BEHAN,I., McMEANS,E. 1951. Biological utilization of unmodified and modified food starches. *J. Nutr 45: 75-95*

BOROS, D., MARQUARDT, R.R., and GUENTER, W. 1998. Antinutritive compounds in three milling fractions from rye and wheat: Improvement in chicks' performance following the addition of a crude enzyme preparation. *British poultry science*

BRILLOUET, J.M., ROUAU, X., HOEBLER, C., CARRE, B., and LORTA, E. 1988. A new method for the determination of insoluble cell walls and soluble nonstarch

polysaccharides for plant materials. *Journal of Agriculture and Food Chemistry 36: 970-979*

BROZ, J. and FRIGG, M. 1986. Effects of beta-glucanase on the feedings of broiler diets based on barley or oats. *Arch. Gefluegelkd 50: 41-47*

CAMPBELL, G.L. and BEDFORD, M.R. 1992. Enzyme application for monogastric feeds: A review. *Canadian Journal of Animal Science 72: 449-466*

CAMPBELL, G.L.,CLASSEN, H.L., REICHERT, R.D. and CAMPBELL, L.D. 1983. Improvement of the nutritive value for broiler chickens by gamma irradiation-induced viscosity reduction. *British Poultry Science 24: 205-211*

CARRE,B., BRILLOUET,J.M. 1986. Yield and composition of cell wall residues isolated from various feedstuffs used for non-ruminant animals. *J. Sci. Food Agric. 37: 341-351*

CHESSON, A. 1993. Feed enzymes. *Animal feed science and Technology 45, 65-69*

CHOCT, M. and ANNISON, G. 1990. Anti-nutritive activity of wheat pentosans in poultry diets. *British Poultry science 31: 809-819*

CHOCT, M. and ANNISON, G. 1992. Anti-nutritive effect of wheat pentosans in broiler chickens: Roles of viscosity and gut microflora. *British Poultry Science 33: 821-834.*

CHOCT, M., HUGES, R.J., TRIMBLE, R.P., ANGKANAPORN, K., ANNISON, G. 1995. Non-polysaccharide-degrading enzymes increase the performance of broiler chickens fed wheat and low-apparent metabolizable energy. *Journal of Nutrition 125: 485-492*

CLASSEN, H.L., BEDFORD, M.R. 1991. The use of enzymes to improve the nutritive value of poultry feeds. *Recent advances in animal nutrition, In Grimsworthy, P.; Haresigh,W.; Cole, D., ed., Butterworth-Heinernarn, Boston, MA. pp. 71-79*

CLASSEN, H.L., CAMPBELL, G.L. and GROOTWASSINK, J.W.D. 1988. Improved feeding value of Saskatchewan grown barley for broiler chickens with dietary enzyme supplementation. *Canadian Journal of Animal Science 68:1253-1259*

CLASSEN, H.L., CAMPBELL,G.L., ROSSNAGEL, B.G., BHATTY, R. and REICHERT, R.D. 1985. Studies on the use of hulless barley in chick diets: deleterious effects and methods of alleviation. *Canadian Journal of Animal Science, 65: 725-733*

COLLIER, B. and HARDY, B. 1986. The use of enzyme in pig and poultry feeds. Part 2. Results of animal trials. *Feed Compounder, 6: 28-30*

COWAN, W.O., JORGENSEN, O.B., RASMUSSEN, P.B. and WAGNER, R. 1993. Role of single xylanase enzyme components in improving feed performance in wheat based poultry diets. *Agro-Food-Industry-Hi-Thch. July/Aug, 11-14*

COWAN, W.O., KORSBAK, A., HASTRUP, T. and RASMUSSEN, P.B. 1994. Influence of added microbial enzymes on energy and protein availability of selected feed raw materials. *Proceedings of the XV Western Nutrition Conference, Winnipeg, Manitoba, pp.143-152*

CROUCH, A.N., GRIMES, J.L., FERKET, P.R. and THOMAS, L.N. 1997. Enzyme supplementation to enhance wheat utilization in starter diets for broiler and turkeys. *Journal of Apply Poultry Research 6: 147-154*

DE SILVA,S., HESSELMAN K. and AMAN,P. 1983. Effects of water and β-glucanase treatment on non-starch polysaccharides in endosperm of low and high viscous barley. *Swedish Journal of Agriculture 13: 211-219*

DUSEL,G., KLUGE, H. and JEROCH, H. 1998. Xylanase supplementation of wheat-based rations for broiler: Influence of wheat characteristics. *Journal of Apply Poultry Research 7: 119-131*

EDNEY, M.J., CAMPBELL, G.L. and CLASSEN, H.L. 1989. The effect of β-glucanase supplementation in nutrient digestibility and growth in broilers given diets containing barley, oats, groats or wheat. *Animal Feed Science Technology 25:193-200*

ENGLYST, H.N. and CUMMINGS, J.H. 1984. Simplified method for the measurement of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as alditol acetates. *Analyst 109: 937-942*

FEIGHNER, S.D. and DASHKEVIEZ, M.P. 1988. Effect of dietary carbohydrates on bacterial cholytaurine hydrolase in poultry intestinal homogenates. *Applied and Environmental Microbiology 54: 337-342*

FENGLER, A.I. and MARQUARDT, R.R. 1988a. Water-soluble pentosans from rye: I. Isolation, partial purification and characterisation. *Cereal Chemistry 65: 291-297*

FENGLER, A.I. and MARQUARDT, R.R. 1988b. Water-soluble pentosans from rye: II. Effects of rate of dialysis on the retention of nutrients by the chick. *Cereal Chemistry 65: 298-302*

FERKET, P.R. 1993. Practical use of feed enzymes for turkeys and broilers. *J. Appl. Poultry Res. 2:75-81.*

FINCHER, G. B. and STONE, B. A. 1986. Cell walls and their components in cereal grain technology. *Advances in Cereal Science and Technology. In: Y.Pomeranz (Editor), Am. Assoc. Cereal Chemistry, MN, pp. 207-293*

FRIESEN, O.D., GUENTER, W., MARQUARDT, R.R. and ROTTER, B.A. 1992. The effect of enzyme supplementation on the apparent metabolizable energy and nutrient digestibilities of wheat, barley, oats and rye for the young broiler chick. *Poultry Science 71:1710-1721*

GHAZI, S., ROOKE, J.A., GALBRAITH, H. and MORGAN, A. 1996b. The effect on nitrogen digestibility in growing chicks and broilers of treating soya-bean meal with different proteolytic enzymes. *Proceedings WPSA spring meeting, U.K. Branch, Scarborough, vol. I, pp:42-43*

GHAZI, S., ROOKE, J.A., GALBRAITH, H. and MORGAN, A. 1996a. The potential for improving soya-bean meal in diets for chicks; treatment with different proteolytic enzymes. *Proceedings WPSA spring meeting, U.K. Branch, Scarborough, vol. I, pp: 40-41*

GOHL, B., ALDEN, S., ELWINGER, K. and THOMKE, S. 1978. Influence of β-glucanase on feeding value of barley for poultry and moisture content of excreta. *British Poultry Science 19:41-47*

GRAF, E. 1986. Chemistry and application of phytic acid: In E. Graf (Editor), *Phytic Acid: Chemistry and Application. Pilatus Press, Minneapolis, MN, pp 1-21.*

GRAHAM, H. and PETTERSSON, D. 1992. A note on the effect of a beta-glucanase and a multi-enzyme on production in broiler chicks fed a barley-based diet. *Swed. J. Agric. Res. 22: 39-42*

GRAHAM, H., FADEL, J.G., NEWMAN, C.W. and NEWMAN, R.K. 1989. Effect of pelleting and β-glucanase supplementation on the ileal and faecal digestibility of a barley-based diet in the pig. *Journal of Animal Science, 67:1293-1298*

GROOTWASSINK, J.W.D., CAMPBELL, G.L. and CLASSEN, H.L. 1989. Fractionation of crude pentosanase (arabinoxylanase) for improvement of the nutritional value of rye diets for broiler chickens. *Journal of Science and Food Agriculture 46: 289-300*

GUENTER,W., SLOMINSKI,B.A., SIMBAYA,J., MORGAN, A. and CAMPBELL, L.D. 1995. Potential for improved utilization of canola meal using exogenous enzymes. *Proceedings of the 9th international rapeseed congress, Cambridge, U.K., pp: 164-166*

HANG, W. And H.J. LANTZSCH, H.J. 1983. Sensitive method for the rapid determination of phytate cereals and cereal products. *J. Sci. Food Agric. 34: 1423-1426.*

HEGER, J., SALEK, M. and EGGUM, B.O. 1990. Nutritional value of some Czechoslovak varieties of wheat, triticale and rye. *Animal Feed Science and Technology 29:89-100*

HENRY, R.J. 1985. A comparison of the non-starch carbohydrates in cereal grains. *Journal of Food Science and Agriculture 36:1243-1243*

HENRY, R.J. 1987. Pentosan and 1-3,1-4 β-glucan concentrations in endosperm and whole grain of wheat , barley, oats and rye. *Journal of cereal chemistry 6: 253-258.*

HEPNER, L. 1995. Commercial status of feed enzymes. *Proceedings of the 2nd European Symposium on Feed Enzymes (W. van Hartingsveldt, M. Hessing, J.P. van der Lugt and W.A.C. Somers, eds) pp. 31-34*

HESSELMAN, K. 1983. Effects of β-glucanase supplementation to barley based diets for broiler chickens. *Report 112, Sveriges Lantbruksunivesitet, Uppsala*

HESSELMAN, K. and AMAN, P. 1986. The effect of β-glucanase on the utilization of starch and nitrogen by broiler chickens fed on barley of low-or high-viscosity. *Animal Feed Science and Technology, 15: 83-93*

HILL, F.W., ANDERSON,D.L., RENNER, R. and CAREW, L.B.Jr. 1960. Studies on the metabolizable energy of grain and grain products for chickens. *Poult. Sci., 39(3): 573-579.*

IKEDA, K. and KUSANA, K. 1983. In vitro inhibition of enzymes by indigestible polysaccharides. *Cereal Chemistry 60:260-263*

INBORR, J. and BEDFORD, M.R. 1994. Stability of feed enzymes to stam pelleting during feed processing. *Animal feed science and technology 46: 179-196*

JANSSEN, W.M.M.A., TERPATRA, K., BEEKING,F.F.E. and BISALSKY,A.J.N. 1979. Feeding values for poultry, *2nd edition, Spelderholt Institute for Poultry Research, Beekbergen, The Netherlands*

JENSEN, L.S., FRY, R.E., ALLRED,J.B. and McGLINNIS, J. 1957. Improvement in the nutritional value of barley for chicks by enzyme supplementation. *Poultry Science 36: 919-921*

JIN, S.H.,CORLESS,A. and SELL,J.L. 1998. Digestive system development in post-hatch poultry. *World's poultry science journal vol. 54:335-345*

JORGENSEN, H., ZHAO, X., BACH KNUDSEN, K.E., EGGUM, B.O. 1996. The influence of dietary fibre source and level on the development of the gastrointestinal

tract, digestibility and energy metabolism in broiler chickens. *British Journal of Nutrition 75: 379-395*

KROGDAHL, A. and SELL, J.L. 1989. Influence of age on lipase, amylase and protease activities in pancreatic tissue and intestinal contents of young turkeys. *Poultry Science 68: 1561-1568*

LESSON, S., YERSIN, A. and VOLKER, L. 1993. Nutritive value of 1992 corn crop. *Journal of Applied Poultry Research 2: 208-213*

LIANG, C., and LIU, Y. 1998. AME examined in relation to wheat-fed broilers. *Feedstuffs October 5: 12-15*

MAENZ, D.D., ENGELESCHAAN,C.M., CLASSEN, H.L. 1997. Endogenous phytase activity in the small intestinal brush border membrance of broiler chicks and laying hens. *In: 86th annual meeting of poultry science association. Vol: 76 s1 p: 281.*

MARCHAIN, U. and KULKA, R.G. 1967. The no-parallel increase of amylase, chymotrypsinogan and procarboxypeptidase in the developing chick pancreas. *Biochimica et. Biophysica Acta 164: 553-559*

MARQUARDT, R.R., BOROS, D., GUENTER, W., CROW, G. 1994. The nutritive value of barley, rye, wheat and corn for young chicks as affected by use of a *Trichodema reesei* enzyme preparation. *Feed Science and Technology 45: 363-378*

McCRACKEN, K.J., URQUHART, R. and BEDFORD, M.R. 1993. Effect of heat treatment and feed enzyme supplementation on the performance of growing broiler chicks. *Proceeding of the Nutrition Society 52:50*

McNAB, J.M. 1996. Factors affecting the energy value of wheat for poultry. *World's Poultry Science Journal, Vol.52, March: 68-73*

MOLLAH,Y., BRYDEN, W.L., WALLIS, I.R., BALNAVE, D. and ANNISON, E.F. 1983. Studies on low metabolizable energy wheats for poultry using conventional and rapid assay procedures and the effects of processing. *British Poultry Science 24: 81-89*

MORAN, E.T., LALL, S.P. and SUMMERS, J.D. 1969. The feeding value of rye for the growing chick: Effect of enzyme supplements, antibiotics, autoclaving and geographical area of production. *Poultry Science 48: 939-949*

MORRIS, E.R. and ROSS-MURPHY, S.B. 1981. Chain flexibility of polysaccharides and glycoprotein from viscosity measurements. *Techniques in carbohydrate metabolism B 310: 1-46*

MULDER,M.M., LOMAX, J.A., HOTTEN, P.M., COWIE, E. and CHESSON,A. 1991. Digestion of wheat aleurone by commercial polysaccharides. *Animal Feed Science Technology 32: 185-192.*

MURISON, S.D., MULDER,M.M. and HOTEEN, P.M., 1989. Enzymatic solubilisation of aleurone cell walls and release of protein. In: S.C. Fry, C.T. Brett and J.S.G. Reid (Editors), Proc. 5th Cell Wall Meeting, Edinburgh, 30 August-2 September 1989. University of Edinburgh. Edinburgh, p. 197.

NEWMAN, R. and NEWMAN, C. 1987. Beta-glucanase effect on the performance of broiler chicks fed covered and hulless barley isotypes having normal and waxy starch. *Nutrition Rsp. Int. 36: 693-699*

NITSAN, Z., DUNTIGTON, E.A. and SIEGEL, P.B. 1991. Organ growth and digestive enzyme levels to fifteen days of age in lines of chickens differing in body weight. *Poultry Science 70: 2040-2048*

NOY, Y. and SKLAN, D. **1995.** Digestion and absorption in the young chick. *Poultry Science 74: 366-373*

NOY, Y. and SKLAN, D. **1997.** Posthatch development in poultry. *Journal of Applied Poultry Research 6: 344-354*

NRC. 1994. Nutrient Requirements of Poultry (9th Ed.). National Academy Press, Washington, DC.

PACK, M., BEDFORD, M. and ROSTAGNO,H.S. **1997.** Effects of feed enzymes on ileal digestibility of energy and protein in corn-soybean diets fed to boilers. *Proceedings of the 11nd European Symposium on Poultry Nutrition. Faaborg, Denmark, 24-28 Augest 1997, 502-504*

PACK, M., BEDFORD, M. and WYATT, C. **1998.** Feed enzymes may improve corn, soryhum diets. *Feedstuffs, February 2, pp:18-19*

PETTERSON, D. and AMAN, P. **1988.** Effects of enzyme supplementation of diets based on wheat, rye, or triticale on their productive value for broiler chickens. *Animal Feed Science and Technology 20: 313-324*

PETTERSON, D. and AMAN, P. **1989.** Enzyme supplementation of a poultry diet containing rye and wheat. *British Journal of Nutrition 62: 139-149*

PETTERSON, D., GRAHAM, H. and AMAN,P. 1987. The productive value of whole and dehulled oats in broiler chicken diets, and influence of β-glucanase supplementation. *Nutrition Reports International 36: 743-750.*

PETTERSON, D., RAHAM, D.H. and AMAN, P. 1991. The nutritive value for broiler chickens of pelleting and enzyme supplementation of a diet containing barley, wheat, and rye. *Animal feed science technology 33: 1-14*

PHILIP, J.S., GILBERT, H.J. and SMITHARD, R.R. 1995. Growth, viscosity and beta-glucanase activity of intestinal fluid in broiler chickens fed on barley-based diets with or without exogenous beat-glucanase. *British Poultry Science 36: 599-603*

RENNER, R. and HILL, F.W. 1960. The utilization of corn oil, lard and tallow by chicken of various ages. *Poultry Science 39: 849*

RICKES, E.L., MOSCATELLI, D.A., and OTTO, W.H. 1962. The isolation and biological properties of a β-glucanase from B.subtilis. *Archives of Biochemistry and Biophysics 96:371-375*

ROGEL, A.M., ANNISON, E.F., BRYDEN, W.I. and RALNAVE, D. 1987. The digestion of wheat starch in broiler chickens. *Aust. J. Agri. Res. 38: 639-649*

ROTTER, B.A., FROHLICH, A.A., ROTTER, R.G. and MARQUARDT, R.R. 1989. Estimation of apparent protein digestibility using uric acid-corrected nitrogen value in poultry excreta. *Poultry science 68: 327-329.*

ROTTER, B.A., MARQUARDT, R.R., GUENTER, W.,BILIADERIS,C. and NESKAR, M., 1989c. In vitro viscosity measurements of barley extracts as predictors of growth responses in chicks fed barley-based diets supplemented with a fungal enzyme preparation. *Can. J. Anim. Sci., 69: 431-439.*

ROTTER, B.A., NESKAR, M., GUENTER, W. and MARQUARDT, R.R. 1989b. Effects of enzyme supplementation on the nutritive value of hulless barley in chicken diets. *Animal Feed Science Technology 24: 233-245.*

ROTTER, B.A., NESKAR, M., MARQUARDT, R.R. and GUENTER, W. 1989a Effects of different enzyme preparations on the nutritional value of barley in chicken diets. *Nutrition research Int. 39: 107-120*

SAKOMURA, N.K., ZANELLA, I., PACK, M., SALNOVA, M.S., ROSA, A.P. and MAGON, L. 1998. Effect of soybean processing and enzyme addition on nutrient digestibility in corn-soybean broiler diets. *Proceedings of Southern Poultry Science Symposium. Jan. 1998, Atlanta, Ga.*

SALIH, M.E., CLASSEN, H.L. and CAMPBELL, G.L. 1991. Response of chickens fed barley to dietary β-glucanase at different ages. *Animal Feed Science and Technology 33:139-149*

SAMMAN, S. and ANNISON, G. 1993. Dietary fibre and beyond Australian perspectives. *Nutrition society of Australia.*

SAULNIER, L., PENEAU, N. and THIBAULT, J.F. 1995. Variability in grain extract viscosity and water soluble arabinoxylan content in wheat. *Journal of Cereal Science 22:259-264*

SCHEIDELER, S.E., IVUSIC, S.I. and AL-BATSHAN, H. 1992. Effect of phytase supplementation on mature weight broilers. *Poultry Science 71(1): 176*

SELVENDRAN, R.R., 1984. The plant cell wall as a source of dietary fibre: chemistry and structure. *Am.J.Cli. Nutr. 39: 320-337.*

SELVENDRAN, R.R., STEVENS, B.J.H. and DU PONT, M.S. 1987. Dietary fiber: Chemistry, analysis and properties. *Advances in food research 31: 117-209*

SILVERSIDES, F.G. and BEDFORD, M.R. 1999. Enzymes may improve energy, protein digestibility. *Feedstuffs, Match, pp: 15-17*

SIMBAYA, J., SLOMINSKI, B.A., GUENTER, W., MORGAN, A., CAMPBELL, L.D. 1996. The effects of protease and carbohydrase supplementation on the nutritive

value of canola meal for poultry: In vitro and in vivo studies. *Animal Feed Technology 61: 219-234*

SLOMINSKI, B.A. and CAMPBELL, L.D. 1990. Non-starch polysaccharides of canola meal: Quantification, Digestibility in poultry and potential benefit of dietary enzyme supplementation. *Journal of Science of Food and Agriculture 53: 175-184*

SLOMINSKI, B.A. and CAMPBELL, L.D. 1991. The carbohydrate content of yellow-seeded canoal. *Proceedings of the 8th International Rapeseed Congress. pp:1402-1407*

SLOMINSKI, B.A. and CAMPBELL, L.D. and GUENTER, W. 1992. Enhancement of the feeding value of low-glucosinolate rapeseed by the supplementation of poultry diets with exogenous enzymes. *Proceedings of the 19th World's Poultryr Congress, Amsterdam, pp: 241-245*

SLOMINSKI, B.A. CYRAN, M. GUENTER, W. and CAMPBELL, L.D. 1999. Nutritive value of enzyme-treated canola meal.

SLOMINSKI, B.A., J. SIMBAYA,J., L.D. CAMPBELL,L.D., G. RAKOW,G. and W. GUENTER,W. 1999. Nutritive value for broilers of meals derived from newly developed varieties of yellow-seeded canola. *Anim. Feed Sci. Technol.(in press).*

SMITS, C.H.M. and ANNISON, G. 1996. Non-starch plant polysaccharides in broiler nutrition- towards a physiologically valid approach to their determination. *World's Poultry Journal, Vol 52, July 203-221*

STATISTITICAL ANALYSIS SYSTEMS INSTITUE. 1985. SAS users guide: Statistics. SAS Institute, Cary, NC, U.S.A.

STEENFELDT, S., ANETTE, M.and JENSEN, F. 1998a. Enzyme supplementation of wheat-based diets for broilers 1. Effect on growth performance and intestinal viscosity *Animal Feed Science and Technology 75: 27-43*

STEENFELDT, S., ANETTE, M.and JENSEN, F. 1998b. Enzyme supplementation of wheat-based diets for broilers 1. Effect on apparent metabolisable energy content and nutrient digestibility *Animal Feed Science and Technology 75: 45-64*

TEITGE, D. A., CAMBELL, G. L., CLASSEN, H. L. and THACKER, P. A. 1991. Heat pre-treatment as a means of improving the response to dietary pentosanase in chicks fed rye. *Canadian Journal of Animal Science 71: 505-513*

TERVILA-WILE, A., PARKKONENT, T., MORGAN, A., HOPEAKOSKI-NURMINEN, M., POUTANEN, K., HEIKKINEN, P. and AUTIO, K. 1996. In vitro digestion of wheat microstructure with xylanase and cellulase from Trichoderma reesei. *Journal of cereal science 24: 215-225*

TOVAR, J., FRANCISCO, A., BJORK, I. and ASP, N. 1991. Relationship between microstructure and in vitro digestibility of starch in pre-cooked leguminous seed flours. *Food Structure 10: 19-26*

TROWELL, H.C., SOUTHGATE,D.A.T., WOLEVER, T.M.S., LEEDS,A.R., GASSULL,M.A. and JENKINS, D.J.A. 1976. Dietary fibre redefined. Lancet. 1: 967. *Animal Journal of clinic Nutrition 25: 926*

UNI, Z., NOY, Y. and SKLAN, D. 1995. Posthatch changes in morphology and function of the small intestine in heavy- and light-strain chicks. *Poultry Science 74:1622-1629*

VAN PARIDON, BOOYMAN,P.A., SELTEN,G.C.M., GEERSE,C., BARUG,D., DEBOT,P.H.M., HEMKE,G. 1992. The application of fungal endoxylanase in poultry diets. *In: Visser,J., Beldan, G., Fusters-van Someren, M.H., Voragen, A.G.J.(Eds). Xylans and Xylanase. Progress in Biochemistry. Elsevier Amsterdam, vol. 7, pp. 371-378*

VANBAARHOVEN, H., ROOKE, J. and MORGAN, A. 1995. Quality of soybean meals and effect of microbial enzymes in degrading soya antinutritional compounds (ANC's) using immunochemical, microscopic techniques and in vivo studies. *Proceedings of the 2nd European Symposium on Feed Enzymes, Noordwijkernout, the Netherlands, pp: 176-177*

VELDMAN, A. and VAHL, H.A. 1994. Xylanase in broiler diets with differences in characteristics and content of wheat. *British Poultry Science 35: 537-550.*

WARD, A.T. and MARQUARDT, R.R. 1987. Antinutritional activity of a water-soluble pentosan-rich fraction from rye grain. *Poultry science 66: 1665-1674*

WHITE, W.B., BIRD, H.R., SUNDE, M.L. and MARLETT, J.A. 1983. Viscosity of β-glucan as a factor in the improvement of barley for chicks. *Poultry Science 62:853-858*

WHITE, W.B., BIRD, H.R., SUNDE, M.L., BURGER, W.C. and MARLETT,J.A. 1981. The viscosity interaction of barley β-glucan with *Trichoderma virida* cellulase in the chick intestine. *Poultry Science 60: 1043-1048*

WILLIAMS, C.H., DAVID, D.J. and LISMOA,O. 1963. The determination of chromic oxide in fecal samples by atomic absorption spectrophotometry. *J. Agr. Sci., 59: 381.*

ZANELLA,I., SAKOMURA, N.K., SILVERSIDES, F.G., EIQUEIREDO, A. and PACK, M. 1999. Effect of enzyme supplementation of broiler diets based on corn and soy-beans. *Submitted to poultry science.*

ZATARI, I. and FERKET, P.R. 1990. The effect of enzyme supplementation of corn-soy diets on the performance of broilers. *Poultry Science 69(1): 149*