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ENZYMES *in* POULTRY *and* SWINE NUTRITION

Proceedings of the
First Chinese Symposium on Feed Enzymes,
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Ronald R. Marquardt and Zhengkang Han

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Preface

The use of enzymes as a feed additive has rapidly expanded during the past 10 years. Although the economic and social benefits of enzymes have been well established, more research and development are needed if enzymes are to reach their full potential in the industry. Specifically, more research is needed

- To explore the mechanisms by which enzymes produce their beneficial effects;
- To identify the sites in the gastrointestinal tract where enzymes are most effective; and
- To determine the types and amounts of enzymes required for different classes and ages of poultry and pigs and for a wide spectrum of feedstuffs.

In addition, we need to develop

- Rreliable ways to assess enzyme potency before and after addition to diets;
- Model systems to accurately predict how poultry and pigs will respond to the use of enzymes; and
- Analytical programs to assess the economic and social benefits of enzyme treatments.

The papers presented at this symposium address some of these problems and recommend areas of further research. The papers point out that the use of enzymes in feeds is only in its infancy and that many exciting developments can be expected, particularly with the use of recombinant enzymes for a wide range of animals and animal feedstuffs. Enzymes not only will enable livestock and poultry producers to economically use new feedstuffs, but will also prove to be environmentally friendly, as they reduce the pollution associated with animal production. In addition, as shown in the studies carried out by Dr Han's groups at Nanjing Agricultural University, enzymes are a very useful tool in the study of physiological and metabolic mechanisms in the animal. Such studies will enhance our understanding of the role of dietary enzymes in animal nutrition.

Much of the research presented at this symposium was supported, in part, by the International Development Research Centre (IDRC), Ottawa, Canada, which provided a grant for a joint China–Canada research project aimed at enhancing the nutritive value of feedstuffs used in China's poultry industry. We believe that the project was successful — many agricultural universities in China have initiated studies on the use of enzyme supplements, and most of the industry is now aware of the benefits of using enzymes.

It has been estimated that the overall economic impact of enzymes in China could be hundreds of millions of dollars per year. The publication of these proceedings should help to promote more widespread use of enzymes in China. The proceedings are being published in both English and Chinese, and this is particularly important, as the Chinese

publication will be the first of its kind. The Chinese version will be available from Nanjing Agricultural University; the English version, from IDRC.

We are grateful to all the participants for making this symposium an outstanding event. We especially thank the staff at Nanjing Agricultural University for their assistance with the meeting, ensuring that it went smoothly. Special thanks are due to Mr Zhiqun Zhang for his tireless efforts in making arrangements for the meetings and to Dr Yonggang Liu for his excellent translations. The scenic tour of Nanjing was appreciated by all.

We gratefully acknowledge the following for their support: IDRC; Nanjing Agricultural University, Nanjing, PR China; National Natural Science Foundation of China; University of Manitoba, Winnipeg, Manitoba, Canada; Finnfeeds International Ltd, Marlborough, Wiltshire, United Kingdom; Euro-Asia Center, Singapore; Hoffmann–LaRoche Ltd, Basel, Switzerland, and Singapore; Kemin; Alltech; Degussa; and other companies.

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Remarks by Dr Zhengkang Han at the opening of the Symposium

A famous scholar in ancient China, Meng-tse (Mencius), said, "With friends from distant lands, how happy we are!" On behalf of the Laboratory of Animal Physiology and Biochemistry, Nanjing Agricultural University, I warmly welcome the guests from abroad and friends from different parts of China. I would like to thank the International Development Research Centre (IDRC), in Canada, and the National Natural Science Foundation (NNSF), in China, for supporting this cooperative project on the use of enzymes to enhance the nutritive value of animal and poultry feeds. We believe that feed enzymes will provide great benefits for agriculture in China.

This Chinese–Canadian project has been very successful. We trained several young scientists in Canada, as well as graduate students in China, particularly in the field of poultry research. We also purchased essential equipment for the project and for future studies on the use of enzymes. For 3 years, we worked in cooperation with the laboratory directed by Dr Marquardt and Dr Guenter at the University of Manitoba. We not only had academic exchanges, but we also established friendships. This symposium is an opportunity for those who worked on this cooperative research to present their findings and also for invited specialists from abroad and from China to speak on practical problems related to the use of enzymes in animal nutrition.

A famous Western poet (Horace) once wrote, "He has half the deed done, who has made a beginning." We hope that this symposium stimulates further research on the use of enzymes, not only in China but worldwide. These studies will be enhanced by future cooperative research projects involving the university and industry. In addition, they will help us contribute to the development of the enzyme industry, possibly in joint ventures with established enzyme companies in China and the Asia–Pacific region.

Finally, we thank IDRC and NNSF for their support for this symposium. We also thank FinnFeeds, Hoffman–LaRoche, Kemin, Alltech, Degussa, and other companies for their financial support.

I am pleased to inform you that the proceedings of this symposium are being published in both Chinese and English. I hope that this symposium will be successful.

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Poultry production in China and the potential for using enzyme preparations

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The demand for animal products increases with improvements in living standards. According to the Food and Agriculture Organization of the United Nations, there has been a dramatic increase in the production of animal and poultry products in China over the last 10 years (FAO 1994). In 1994, pork meat production was 18% higher than in 1993; poultry meat production, 13.5% higher; total meat production, 11.3% higher; and egg production, 8.3% higher. Demand is likely to dramatically expand in the future: it is predicted that by 2000 meat production will increase to 10×10^6 t and egg production will increase to 2.5×10^6 t (Simpson and Ward 1995). Currently, China is the world's largest producer of meat products, including pork and eggs, and is the second largest producer, after the United States, of poultry meat. In China, the average annual per capita consumption of pork is 24 kg; of poultry, 4.7 kg; and of eggs, 7.7 kg. This is still not as high as in the developed countries: for example, in the United States the values are 30, 48, and 16.5 kg, respectively. Similar (but not identical) statistics have been reported elsewhere (Ministry of Agriculture 1995).

Poultry products represent a significant proportion of the total meat produced in China, and the amount is increasing at a faster rate than that of meat products. In 1994, poultry meat accounted for 14.5% of total meat production, and this is projected to increase to 20% by 2000. It is evident that poultry production plays an important role in animal husbandry in China.

Intensive poultry production in China is still in its infancy, having only a about a 10-year history. It is, nevertheless, rapidly developing. A production system has been established; for example, 200 farms produce grandparent chickens, and 2 000 farms produce parent chickens. These farms provide the breeding stock for the commercial poultry producers. Three kinds of commercial production systems have been established: chicken factory farms, medium- and small-scale chicken farms, and personal chicken farms. Accordingly, feed manufacturing has developed rapidly, and an integrated production system has been implemented.

The feed manufacturers produce about 310×10^6 t of high-quality feed, saving about 30%, or about 93×10^6 t, of feed. The development of a strong feed industry has greatly facilitated the large increase in poultry production in China.

The poultry industry in China is characterized by lower production efficiency and larger fluctuations than are found in developed countries. In 1992, China's feed industry was at the bottom of the valley, but in 1994 there was a very high rate of development. The depression in 1992 was caused by the short supply of feed. This resulted in an increase in feed prices and a decrease in the production of poultry products. Thus, feed availability is the key factor limiting the development of poultry production. Our task is to strengthen scientific research, promote the spread of new techniques in China, and develop new feed resources to ensure an adequate supply of highly nutritious feeds. The use of enzymes is one of the important new techniques for enhancing the efficiency of feed utilization in China and thus for increasing poultry output per unit of feed.

Potential benefits of using enzymes in China

It is apparent that there is insufficient feedstuff in China. In 1994, per capita grain consumption was 333 kg, with 20% of it being used for feedstuffs. It is predicted that from 2000 to 2020 the deficiency will be $24–83 \times 10^6$ t of feed for energy and $24–48 \times 10^6$ t of feed for protein. There are three ways to solve the problem of feed deficiency: increase the production of grain, enhance the efficiency of feed utilization, and develop new feed resources. The first solution will be difficult to implement, but the second and the third are reasonably practical. Enhanced utilization of feed is one of the more effective and promising approaches.

Corn

Corn is not the dominant cereal in China. Annual production is 70×10^6 t (about 20% of the total grain produced), with production scattered unevenly. An improvement in the availability of phytic phosphorus is urgently needed — corn contains 50–75% of the total phosphorus in the diet, but only 10–12% of it can be used by swine or poultry. Using phytase can considerably improve the efficiency of phosphorus utilization, which not only saves phosphorus but also reduces environmental pollution. Corn also contains small amounts of antinutritive factors, such as xylan and cellulose, which may respond to enzyme treatment.

Protein supplements

In China, 4.7×10^6 t of soybean cake, 5.23×10^6 t of cottonseed cake, and 4.85×10^6 t of rapeseed cake were produced in 1993. The digestibility values for these

supplements are 70, 50, and <50%, respectively. The poor utilization of protein is related to its structure. Enzyme addition should enhance overall amino acid utilization, including that of methionine (10–28%), lysine (10–40%), and cystine (14%). If the efficiency of soybean utilization is increased by 10%, 2×10^6 t of soybean cake can be saved in 1 year. Clearly, the potential benefits are very large.

Cereal grains

The supply of corn is insufficient to meet demand, but large quantities of other cereals are grown in China, including rice, wheat, barley, rye, oats, and sorghum. Rice and barley are mainly produced in southern China; wheat, rye, oats, and sorghum, in northern and northwestern China. However, utilization-efficiency values for these cereals are lower than that for corn because they contain different antinutritive factors, especially the nondigestible polysaccharides (Marquardt et al. 1996). A substantial amount of research in other countries has shown that the utilization-efficiency values for cereals can be improved by adding enzymes. Our trials also indicated that growth rate was significantly increased by adding enzymes to barley-based diets: the improvement was 10% for broilers, 12–18% for ducks, and 10–21% for geese. In some studies, performance of the different classes of poultry fed enzyme-supplemented diets was almost equal to that obtained with corn-based diets. This new technique should help solve the feed-deficiency problem and reduce the scatter of feed energy in China.

Reform of the cultivation system

The addition of enzymes to barley- and wheat-based poultry diets, for example, should increase the demand for and cultivation of these cereal crops. This will in turn facilitate the development of coastal farms with saline soil; the more effective use of soil resources in the northwest; and the use of cereal by-products.

Saline coastal soil is a large resource in China, and barley is a salt-tolerant plant. The newly developed coastal region is well suited for cultivating barley, and barley can be used as an animal feed, especially if enzymes are added to the diet. In addition, barley, with its short growing period, is very important to the reform of the cultivation system in our country.

Northwestern China will also benefit from the use of enzymes. A new variety of spring wheat (called 338) has been cultivated in Qi Hai Province. Its productivity is 77% higher than that of common wheat, but it cannot be used as food because of its poorer quality. Nevertheless, this variety of spring wheat would have great economic value if it could be used as animal feed, especially with the addition of enzymes to the diet. Similar situations undoubtedly exist in other parts of China.

By-products from grain processing are substantial. The annual yield of bran mash and bran flour is 36×10^6 t, which equals 21% of the country's total grain production. A reduction of the negative effects of the antinutritive factors (probably arabinoxylans) in wheat bran, bran flour, and rice bran would provide new feeds for poultry and monogastric animals. Preliminary results have shown that adding enzymes to rice-bran diets increased weight gains by 11%.

Conclusion

As indicated above, the availability of feed is the key factor limiting poultry production. China has abundant feed resources, but these are often of poor quality and are therefore poorly utilized by poultry. The cooperative project undertaken by Canada and China (see the Preface) has resulted in a very effective technique for improving feed efficiency, developing new feed resources, and establishing a stable base for feed production. This technique will greatly benefit our country, as it would other countries of the Third World. We believe that enzyme preparations will have broad applications and a very significant impact on agriculture in China and will provide economic and social benefits, not only to the producers of feed and animal products but also to the population as a whole.

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Enzyme enhancement of the nutritional value of cereals: role of viscous, water-soluble, nonstarch polysaccharides in chick performance

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Enzymes were used in the preparation of foods long before there was any awareness of enzymes as such, possibly as long ago as 10 000 years. The industrial exploitation of microbial enzymes in the Western world started 100 years ago with the patenting of a process for the production of alpha-amylase (Takamine) from the fungus *Aspergillus oryzae*. Most of the enzymes currently used in the food and beverage industry are from *Aspergillus*, but hemicellulases and cellulases are derived from *Trichoderma*. Recently, genes encoding for different enzymes, including phytases, β-glucanases, and xylanases, have been cloned and expressed in different commercial systems (microorganisms and plants).

Some of the enzymes that have been used over the past several years or have potential for use in the feed industry include cellulase (β-glucanases), xylanases and associated enzymes, phytases, proteases, lipases, and galactosidases (Table 1). Enzymes in the feed industry have mostly been used for poultry and, to a lesser degree, for pigs to neutralize the effects of the viscous, nonstarch polysaccharides in cereals such as barley, wheat, rye, and triticale. These antinutritive carbohydrates are undesirable, as they reduce digestion and absorption of all nutrients in the diet, especially fat and protein. Recently, considerable interest has been shown in the use of phytase as a feed additive, as it not only increases the availability of phosphate in plants but also reduces environmental pollution. Several other enzyme products are currently being evaluated in the feed industry, including protease to enhance protein digestion, lipases to enhance lipid digestion, β-galactosidases to neutralize certain antinutritive factors in noncereal feedstuffs, and amylase to assist in the digestion of starch in early-weaned animals.

This review will cover only the use of β-glucanases and xylanases in monogastric diets containing cereal grains such as barley, wheat, rye, triticale, and

Table 1. Some enzymes that are or can be used in the feed industry.

Enzymes	Substrate	Function	Benefits or use
β-glucanases	Barley Oats	Viscosity reduction	Enhanced digestion and utilization of nutrients
Xylanases	Wheat, rye Triticale Rice bran(?)	Viscosity reduction (other effects?)	Enhanced digestion and utilization of nutrients
β-galactosidases	Grain legumes Lupins	Viscosity reduction (other effects?)	Enhanced digestion and utilization of nutrients
Phytases	Plant feedstuffs	Release of phosphate from phytate-P	Enhanced phosphate absorption
Proteases	Proteins	Hydrolysis of protein	Increased digestion of proteins
Lipases	Lipids	Hydrolysis of fats	Use in young animals
Amylases	Starch	Hydrolysis of starch	Supplemental amylase for young animals

oats. Particular emphasis will be on the ability of these enzymes to reduce the viscosity of β-glucans and arabinoxylans present in cereal-based diets and on the role of these enzymes in improving nutrient utilization.

Structure and hydrolysis of β-glucans

β-glucans constitute the most abundant class of naturally occurring polysaccharides because of the wide occurrence of the 1,4-β-glucan, cellulose. However, many other β-glucans are produced by both microbial and nonmicrobial (plant) sources. β-glucans are homopolymers of D-glucose linked in a β-configuration. Some are relatively simple molecules consisting of linear chains of glycosyl residues joined by a single linkage type; others consist of a variety of linkages in either linear or branched chains. Linkage groups include 1,4-β-; 1,3-β-; 1,6-β-; 1,3-β- and 1,6-β-; 1,3-β- and 1,4-β-; and 1,2-β- and 1,4-β-. The (1→3, 1→4)-β-glucans from the cell walls of cereal grains consist of linear chains of β-glycosyl residues joined by both (1→3)- and (1→4)-glycosidic linkages. Further details can be obtained in reviews by Fincher and Stone (1986) and Pitson et al. (1993).

The production of β-glucan-degrading enzymes is a characteristic attributable to a wide variety of organisms, although the fungi are the most common producers of this enzyme. The many β-glucan-hydrolyzing enzymes are classified according to the type of β-glucosidic linkage(s) they cleave and their mechanism of substrate attack (Pitson et al. 1993). A summary of the different β-glucan-degrading enzymes is outlined in Table 2.

Table 2. Nomenclature and action of β-glucan-degrading enzymes.

Code No.	Common name	Systematic name	Action
3.2.1.4	Cellulase	1,4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase	Endohydrolysis of 1,4 linkages in cellulose and β-D-glucans containing 1,3 and 1,4 linkages
3.2.1.6	Laminarinase	1,4-(1,3;1,4)-β-D-glucan 3(4)-glucanohydrolase	Endohydrolysis of 1,3 or 1,4 linkages in β-D-glucans when the glucose residue whose reducing group is involved in the linkage to be hydrolyzed is itself substituted at C-3
3.2.1.21	β-glucosidase	β-D-glucoside glucohydrolase	Hydrolysis of terminal nonreducing β-D-glucosyl residues, with the release of β-D-glucose
3.2.1.39	Endo-1,3-β-glucanase	1,3-β-D-glucan glucanohydrolase	Endohydrolysis of 1,3 linkages in 1,3-β-D-glucans
3.2.1.58	Exo-1,3-β-glucanase	1,3-β-D-glucan glucohydrolase	Exohydrolysis of 1,3 linkages in 1,3-β-D-glucans, with the release of α-glucose
3.2.1.71	Endo-1,2-β-glucanase	1,2-β-D-glucan glucanohydrolase	Endohydrolysis of 1,2 linkages in 1,2-β-D-glucans
3.2.1.73	Lichenase	1,3-1,4-β-D-glucan 4-glucanohydrolase	Endohydrolysis of 1,4 linkages in β-D-glucans containing 1,3 and 1,4 linkages
3.2.1.74	Exo-1,4-β-glucanase	1,4-β-D-glucan glucohydrolase	Exohydrolysis of 1,4 linkages in 1,4-β-glucans
3.2.1.75	Endo-1,6-β-glucanase	1,6-β-D-glucan glucanohydrolase	Endohydrolysis of 1,6 linkages in 1,6-β-glucans

Source: Adapted from Pitson et al. (1993).

Cellulases are the most widely foundβ-glucanases in fungi hydrolyzing the 1,4-β-glucan (cellulose). The 1,3-β- and 1,2-β-glucanases appear to be widely distributed in both fungi and yeast. Although 1,6-β-glucanases are less common than 1,3-β- or 1,4-β-glucanases, they are produced by many fungi. Many workers use the terms *endo-1,3-β-glucanase* and *laminarinase* interchangeably and incorrectly. Endo-1,3-β-glucanases are involved in the hydrolysis of 1,3-β-glucosidic linkages, whereas laminarinases hydrolyze 1,3-β- or 1,4-β-glucosidic linkages when the glucose residue whose reducing group is involved in the linkage to be hydrolyzed is itself substituted at C-3 (Figure 1).

Partial hydrolysis of the β-glucans, particularly by the endo form of the enzyme, has been shown to not only reduce the viscosity of the β-glucans but also to improve the nutritional value of cereals. The exo-β-glucanases, although capable of releasing glucose from β-glucan, have relatively little effect on the viscosity of β-glucans and therefore are of little benefit as an agent for reducing their viscosity. The complete degradation of β-glucans to glucose is accomplished by the synergistic interaction of both endo- and exo-β-glucanases. However, dietary enzymes are, at best, only able to partially hydrolyze β-glucans. Therefore, they probably do not directly increase the availability of glucose from the β-glucans. It is not known whether a similar type of interaction occurs among the various endo-β-glucanases to enhance their ability to reduce the viscosity of β-glucans and therefore to improve the nutritional value of the diet.

Structure and hydrolysis of arabinoxylans

The arabinoxylans extracted from wheat flour are well characterized (Fincher and Stone 1986). They consist predominantly of the pentoses (arabinose and xylose) and hence are often referred to as pentosans. Arabinoxylans are composed of a β-(1→4)-D-xylopyranosyl backbone, with one or more α-L-arabinofuranosyl residues substituted at position 2 or 3 (Figure 2). However, in many instances, hexoses and hexuronic acids are also minor but important constituents. For this reason they are more correctly and descriptively referred to as heteroxylans. In addition, they may contain different phenolic acids, such as ferulic acid and acetyl esters. Figure 3 is a schematic diagram of the major chemical bonds in cell walls and the enzyme cleavage sites. The hemicellulose fraction is composed mainly of xylans that are linked to cellulose fibrils by hydrogen bonds. The xylose residues tend to be acetylated at positions 0–2 and 0–3, whereas other groups, such as ferulic acid, tend to be bound to positions 0–2 or 0–3 and lignin, forming cross-links that entrap the polymer. A combination of enzymes is therefore required for the release of arabinoxylans from the cell matrix and for its hydrolysis (Wuban et al. 1993).

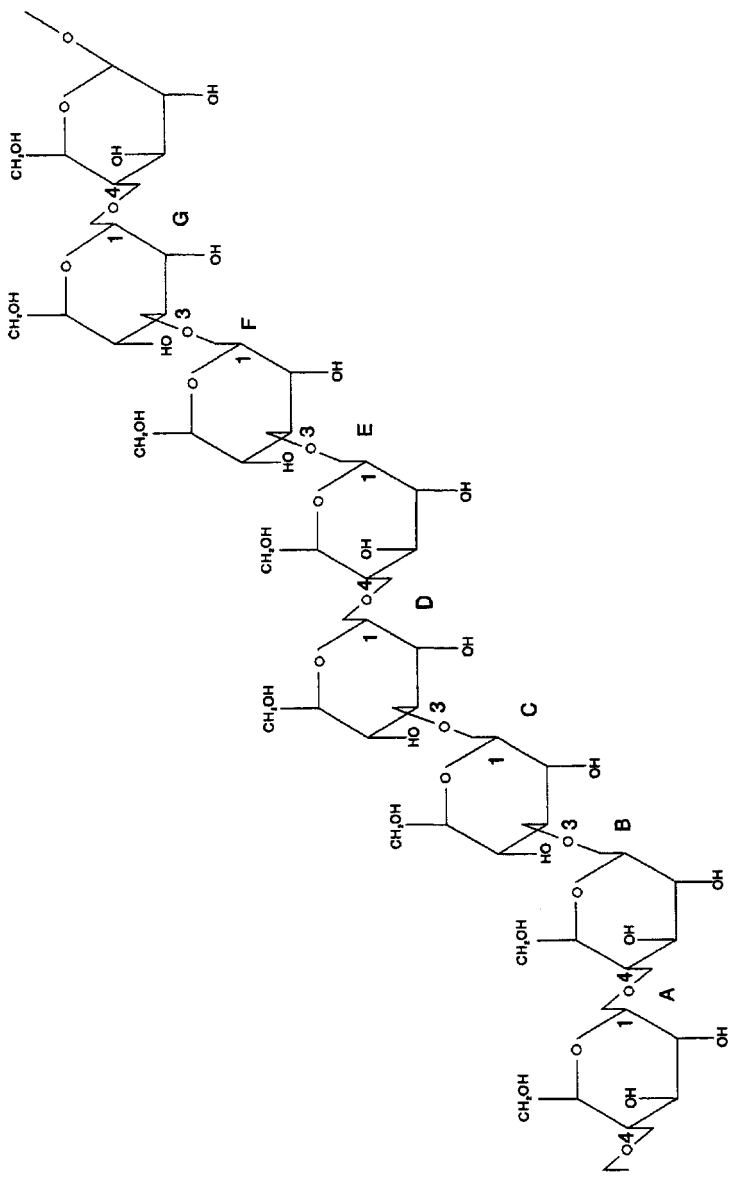

Figure 1. Action of laminarinase and endo-1,3-β-glucanase on a 1,3- or 1,4-β-D-glucan. Laminarinases cleave linkages C, D, F, and G, whereas endo-1,3-β-glucanases cleave linkages B, C, E, and F (Pitson et al. 1993).

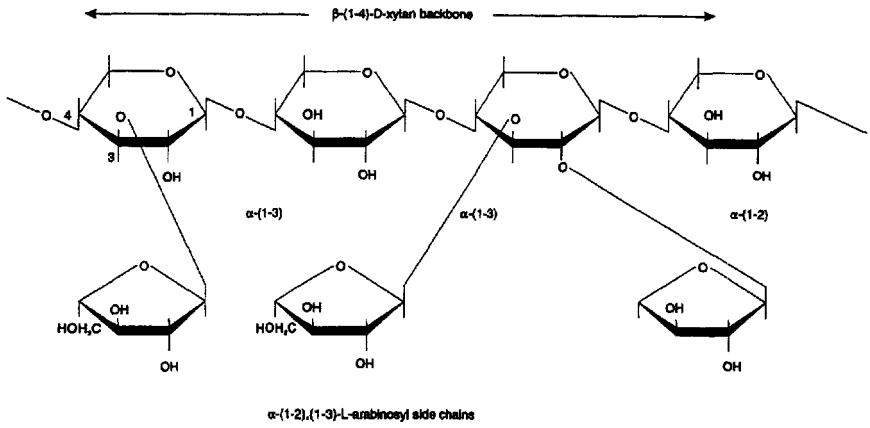

Figure 2. Structure of arabinoxylan {α-(1→2,3)-L-arabinofuranosyl-}"-β-(1→4)-D-xylopyranan, illustrating the main structural features of polysaccharides.

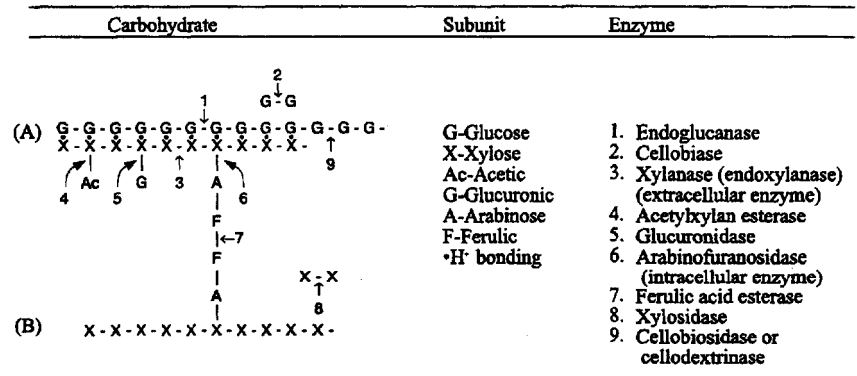

Figure 3. Major chemical bonds of plant cell walls and their cleavage (based on data from Forsberg et al. 1993): (A) β-glucan; (B) pentosan (arabinoxylan). Enzymes 1, 3, and possibly 6 are particularly important for reduction of viscosity in cereals. See Table 2 for more details on the specificity of enzymes 1, 2, and 9.

Physical and chemical properties of water-soluble β-glucans and arabinoxylans

Previous studies have indicated that the viscous, water-soluble β-glucans and arabinoxylans were mainly responsible for the antinutritive properties of wheat, rye, triticale, barley, and oats (Annison and Choct 1991; Campbell and Bedford 1992; Bedford 1995; Jeroch et al. 1995; Marquardt et al. 1996). A comparison of some

Table 3. Physical and chemical properties of water-soluble, nonstarch polysaccharides (WSNSPS) in cereals.

Property	Wheat	Rye	Triticale	Barley	Oats
Yield of WSNSPS (%) [a]	0.6	1.7	0.7	1.2	1.0
Monosaccharide (mg/g) [b]					
Arabinose	238	254	281	66	81
Xylose	264	364	267	75	63
Glucose	43	55	34	481	443
Others	135	46	91	29	53
Total	680	719	673	651	640
M_w [c]	255 000	770 000	569 000	665 000	446 000
M_n [d]	60 500	90 300	66 000	89 700	66 500
Polydispersity (M_w/M_n)	4.2	8.5	8.6	7.4	6.7
Viscosity, η (dL/g) [e]	1.7	5.9	4.0	4.5	3.1
Water-binding capacity (g water/g dry sample)	0.41	0.47	0.42	0.49	0.44
Relative growth depression [f]	+	+++	+(+)	++	++

Source: Adapted from Girhammar and Nair (1992).

[a] WSNSPS in wheat, rye, and triticale are mainly arabinoxylans, whereas those in barley and oats are mainly β-glucans.

[b] Control monosaccharides per gram of extract after hydrolysis.

[c] Weight-average molecular weight.

[d] Number-average molecular weight.

[e] Intrinsic viscosity.

[f] Relative growth depression associated with the feeding of the particular cereal and, more specifically, its WSNSPS.

of these properties is given in Table 3. The water-soluble, nonstarch polysaccharides (WSNSPS) of wheat, rye, and triticale are mainly pentosans, whereas those of barley and oats are mainly the β-glucans. There appears to be a good association between the growth-depressing effect of the cereals and their content of viscous WSNSPS. This is clearly indicated by comparing wheat and rye.

Specifically, there appears to be a high correlation between the amount of soluble pentosans or β-glucans in cereals and extract viscosity. Bengtsson et al. (1992) found that the type of pentosan may also affect viscosity. These researchers isolated two types of pentosan, arabinoxylans I and II, from rye. Arabinoxylan I contains terminal arabinose and unsubstituted and mono-substituted xylose residues, whereas arabinoxylan II contains terminal arabinose and unsubstituted and double-substituted xylose residues (Figure 4). Bengtsson et al. also observed that

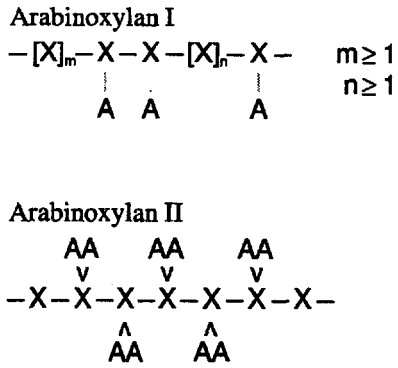

Figure 4. Structural features of arabinoxylan I and II in rye grain (Bengtsson et al. 1992): *A*, terminal α-L-arabinofuranosyl; *X*, 4-linked β-D-xylopyranosyl residues.

arabinoxylan II was considerably more viscous than arabinoxylan I and that the content of the two varied among varieties of rye. Similar variations in pentosans in other cereals have also been reported (Fincher and Stone 1986). Form II, in addition to having a different viscosity, is less readily hydrolyzed by xylanase than form I because of steric hindrance by the arabinose side chains. The accessibility of xylanase to the xylan backbone would presumably be improved if the arabinose is removed first from the pentosan by the action of arabinofuranosidase, another enzyme. A similar situation may also occur when cereals are fed to animals, with the effect of xylanase being enhanced by the presence of arabinofuranosidase.

A considerable degree of variation in the contents of soluble β-glucans and arabinoxylans has also been reported. This occurs not only among the different cereals but also among varieties within a given cereal and even among individuals of the same variety. The content of the viscous carbohydrates in a given cereal varies with method of isolation, variety, soil and climate, agronomic practices, degree of maturity at harvest, storage conditions, and so on (Henry 1986; Saastamoinen et al. 1989). The content of soluble β-glucans, for example, is much higher in Scout, a hull-less barley, than in Bedford, a hulled barley (Rotter et al. 1989). It is also known that maturation of barley and rye under dry growing conditions is associated with an increased concentration of viscous carbohydrates. In addition, the viscosity of stored grain tends to be less than that of fresh grain, perhaps as a result of the activity of endogenous enzymes. The viscosities of aqueous extracts of different cereals are therefore highly variable as a result of variations in the amount of soluble β-glucans or arabinoxylans, which in turn is affected by genetic and environmental factors. Cereals that have a high concentration of these factors are poorly utilized by poultry and presumably by other monogastric animals. The negative effects, however, can be neutralized by the use of enzymes that partially hydrolyze the viscous carbohydrates.

Table 4. Some benefits obtained from the addition of enzymes to poultry feeds.

Reduced viscosity in the diet and digesta
Enhanced digestion and absorption of nutrients especially fat and protein
Improved AME value of the diet
Increased feed intake, weight gain, and feed–gain ratio
Reduced beak impaction and vent plugging
Decreased size of gastrointestinal tract
Altered population of microorganisms in gastrointestinal tract
Reduced water intake
Reduced water content of excreta
Reduced production of ammonia from excreta
Reduced output of excreta, including reduced N and P
Reduced output of bile salts in digesta

Note: For further information, see reviews by Annison and Choct (1991), Campbell and Bedford (1992), Bedford (1995), and Marquardt et al. (1996), as well as papers in these proceedings. AME, apparent metabolizable energy.

Improvements obtained with different enzyme preparations

Enzymes added to poultry diets, especially diets containing cereals such as wheat, barley, and rye, not only enhance the nutrient availability of these diets but also produce many other benefits (Table 4). Some of the other benefits are discussed in another paper in these proceedings (see Choct, this volume).

The degree of improvement obtained by adding enzymes to the diet depends on many factors, including the type and amount of cereal in the diet; the level of antinutritive factor in the cereal, which can vary within a given cereal (for example, low- versus high-β-glucan barley); the spectrum and concentration of enzymes used; the type of animal (poultry tend to be more responsive to enzyme treatment than pigs); and the age of the animal (young animals tend to respond better to enzymes than older animals).

Table 5 shows the improvements obtained in growing chicks when five different cereals were supplemented with an enzyme preparation high in xylanase and β-glucanase activities. In general, there is a close relationship between the amount of soluble arabinoxylans or β-glucans in the different cereals, their corresponding viscosities, and the performance of the chicks. For example, naked oats, being very high in viscous β-glucans, resulted in the greatest degree of response to enzyme treatment. Enzyme treatment improved weight gain by 46% (100 g/bird

Table 5. Effect of enzyme supplementation of five different cereal-based diets on the performance of Leghorn or broiler chicks.

				Barley		
	Corn [a]	Wheat [a] (Katepwa)	Rye [a] (Prima)	Hulled [b] (Bedford)	Hull-less [b] (Scout)	Oats [c] (Terra)
Relative content of soluble arabinoxylans	0	+	++++	0	0	0
Relative content of soluble β-glucans	0	0	0	+	++++	++++
Relative viscosity	0	+	++++	+	++++	++++
			Effect of enzyme (% of the results obtained with control diet)			
Weight gain	102	110	124	113	149	146
Feed–gain ratio	100	96	85	96	75	84
AME_N of diet	99	104	110	106	142	133
APD of diet	98	102	106	105	129	123
Lipid digestibility	—	—	—	113	185	293
Water content of excreta	98	88	93	—	—	—
Gizzard weight	—	—	—	92	83	—
Jejunum length	—	—	—	91	78	—

Note: Relative contents of β-glucans and arabinoxylans and the corresponding viscosity of the cereals are indicated by the number of plus signs. AME_N, apparent metabolizable energy corrected for nitrogen; APD, apparent protein digestibility.

[a] Data from Marquardt et al. (1994). Enzyme was high in xylanase and β-glucanase activities.

[b] Data from Brenes et al. (1993). Enzyme was high in xylanase and β-glucanase activities.

[c] Data from Friesen et al. (1992). Enzyme was high in xylanase and β-glucanase activities.

without treatment versus 146 g/bird with treatment); apparent metabolizable energy (AME), by 33% (10 786 kJ / kg of diet versus 14 327 kJ / kg of diet); and fat digestibility, by 193% (17.4% versus 51%). In contrast, adding enzymes to a corn-based diet had no effect on chick performance. Corn, as indicated in Table 5, essentially does not contain viscous WSNSPS, so therefore no response to enzyme supplementation would be expected. A decrease in size of the intestine following enzyme addition is presumably related to a more efficient and rapid digestion of nutrients, reducing the need for an enlarged intestine. Reduced water intake (data not shown) and water content of the excreta are probably related to a reduced ability of the partially hydrolyzed WSNSPS to absorb water. The latter effect is of considerable importance, as wet excreta is associated with vent pasting; soiled birds and eggs; enhanced ammonia production (due to continued fermentation by microorganisms); and an enhanced load of fungal spores, including those that may be responsible for damage to farmers' lungs (aspergillosis).

These observations indicate that enzyme supplementation of cereal-based diets can have a dramatic effect on the performance and probably also the physiological responses of chickens. These responses are discussed elsewhere in these proceedings (see Han, this volume).

Conclusion

Added enzymes can enhance the nutritional value of poultry diets. The effect on chick performance depends on the type of antinutritive factor present in the diet, the nature of other ingredients, the type and amount of enzymes used, and the age of the animal. The nutritional value of cereals that contain a high level of viscous WSNSPS can usually be improved to a marked degree by the addition of the appropriate enzyme to the diet. In general, barley and oats have a high content of soluble β-glucans and therefore respond to β-glucanase, whereas wheat and rye tend to have higher contents of arabinoxylans and therefore respond to xylanases and possibly other associated enzymes. Corn, in contrast, contains very low levels of WSNSPS; therefore, nutrient utilization is not depressed, and as a result, corn does not show a response to enzyme treatment. Enzyme addition to a diet high in WSNSPS has been shown to improve weight gain, feed–gain ratio, apparent protein digestibility, apparent lipid digestibility, and the AME of the diet. In addition, enzymes reduce the size of the gastrointestinal tract, the water content of excreta, and the incidence of vent pasting. When properly used, enzymes can produce many beneficial effects in the chicken and other kinds of poultry and monogastric animals, such as the young pig.

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Reduced viscosity of intestinal digesta and enhanced nutrient digestibility in chickens given exogenous enzymes

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Many of the grains used in poultry diets are considered potentially viscous, but cereals such as maize and sorghum do not seem to pose much of a problem (Bedford 1996, table 1). Viscosity is a result of the dissolution of large-molecular-weight nonstarch polysaccharide complexes from the endosperm cell walls of the cereals concerned. By their own right, these compounds increase the viscosity of the solution, but at critically high concentrations they can aggregate, forming macromolecular complexes (Annison 1995) that massively increase solution viscosity. These soluble compounds are primarily arabinoxylans and β-glucans. The former are most important in wheat, rye, and triticale; the latter, in barley and oats.

Viscosity in the intestine may pose a problem through two direct mechanisms:

1. As viscosity increases, the rate of diffusion of solutes is reduced (Fengler and Marquardt 1988; Bedford 1996). This was originally thought to be relevant only to high-viscosity solutions, but the work of Bedford (1996) indicated that the rate of diffusion of Bradykinin, a protein of molecular weight 1 000, was reduced by a factor of 40% when solution viscosity was raised from 1 mPa · s to only 5 mPa · s by the use of wheat arabinoxylans. This effect evidently slows the rate of digestion and hence the rate of nutrient extraction from the diet. The larger the molecule is, the greater the impact of highly viscous gels on the rate of diffusion. Given the range of viscosities encountered at the intestinal level for various cereals (Table 1), it seems that diffusion may limit feed efficiency, even in low-viscosity grains like maize and sorghum.

Table 1. Mean, minimum, and maximum intestinal viscosities determined in 21-d-old broilers.

	Intestinal viscosity (cP) [a]		
Cereal	Minimum	Maximum	Mean
Maize	1.5	4.5	2.4
Wheat	3	45	12
Triticale	5	40	16
Barley	6	225	25
Rye	70	>1 000	>250

Source: Bedford (1966).
[a] 1 P = 0.1 Pa · s.

2. As viscosity increases, the ability of the gut to physically mix the contents is severely compromised (Edwards et al. 1988). This is likely to have severe implications for fat digestion because vigorous mixing is required for good emulsification, which is a prerequisite for efficient fat digestion. This is certainly borne out by the results of Dänicke et al. (1995), who found that the digestion of tallow (which is more dependent on emulsification for digestion) was far more severely compromised than that of soybean oil in a highly viscous rye-based diet.

The indirect effects of high digesta viscosity are covered in another paper in these proceedings (Choct, this volume), so they will not be covered in detail here. Nevertheless, there are indications that high digesta viscosity affects performance by altering gut-enterocyte turnover rates, endogenous-enzyme synthesis rates, microfloral and coccidial populations, and litter quality (Choct et al. 1995; Morgan and Bedford 1995; Smithard and Silva 1996).

Exogenous enzymes used in viscous grain diets function by simply breaking up the structure of the soluble, viscous gel. This is theoretically not a difficult task, as only comparatively few enzymatic cleavages are required to significantly damage the nature of the gel. The best strategy is to ensure the enzyme is endo-acting, that is, that it favours binding and cleaving bonds toward the centre, rather than the ends, of the nonstarch polysaccharide molecule. Hence, the most effective enzymes used in poultry feed today are endo-xylanases or endo-ß-glucanases.

Thus, it is evident that increasing digesta viscosity can negatively influence the rate of nutrient digestion and hence subsequent broiler performance, but this view is still not universally accepted. This paper will attempt to clarify this debate and show the benefits of viscosity reduction in terms of nutrient digestibilities and bird performance.

Table 2. Diet formulations used in experiments 1 and 2.

	Content (g/kg)		
	Experiment 1		Experiment 2
Ingredient	Starter	Finisher	Starter
Wheat	616	631	638
Soybean meal, 48%	307	269	243.5
Fishmeal (65% protein)	0.0	0.0	50.0
Soybean oil	34.7	60.4	28.0
Salt	3.8	3.0	0.7
DL isomer of methionine	1.9	0.8	0.6
Limestone	13.8	14.5	4.4
Dicalcium phosphate	12.5	11.8	9.5
Vitamins and minerals	10.0	10.0	5.0

Methods

The two experiments described in this paper generally followed the same protocols. Birds were grown in environmentally controlled cages or floor pens and subjected to 23.5 h of light and 0.5 h of dark / d. Feed and water were offered *ad libitum* (diet formulations are given in Table 2 for the two experiments). For ileal digestibility measurements, the method of Coon et al. (1988) was followed. Starter diets were fed from 0 to 21 d of age, and finisher diets (experiment 1 only) were fed from 22 to 42 d of age.

Experiment 1: dose–response study

Experiment 1 investigated the effects of varying the inclusion level of a xylanase-based enzyme (*Trichoderma longibrachiatum*) in a wheat-based diet to establish a relationship between intestinal viscosity and performance in low-viscosity diets. Six inclusion levels of enzyme, resulting in 0, 690, 1 380, 2 070, 2 760, and 3 450 U xylanase activity / kg feed, were investigated. Gain and intake at 21 and 42 d of age were determined, and resultant feed-conversion rates (FCRs) were calculated for each period. Intestinal viscosity was determined at 21 d of age by the method described by Bedford et al. (1991). Twelve replicate pens of 65 male Ross 1 broilers were offered each of the diets in pelleted form (76°C maximum pellet temperature).

Experiment 2: ileal digestibility study

Experiment 2 investigated the effect of inclusion of 3 000 U xylanase activity / kg feed on gain, intake, calculated FCR, intestinal viscosity, and ileal digestibility of energy, protein, and amino acids in birds fed a diet based on one of two samples of wheat. Thus, four diets in total (wheats 1 and 2 with 0 or 3 000 U xylanase activity / kg feed) were employed. Three replicate cages of 12 birds (Cobb male broilers) per diet were used in the growth period (0–21 d). At 21 d of age, four birds per cage were sacrificed for individual determination of ileal digestibility of energy, protein, and amino acids. Chromic oxide was used as the marker, following the method of Coon et al. (1988).

All data were analyzed according to the general linear model procedure of the SAS Institute, Inc. (1986), and means were separated, where appropriate, by orthogonal contrasts. For experiment 1, regression lines were established by use of orthogonal polynomial contrasts.

Results and discussion

Experiment 1: dose–response study

The data shown in Table 3 clearly indicate that the optimum inclusion level of enzyme in terms of FCR response (FCR is the most sensitive parameter) was greater for older than for younger birds. The fact that as the bird ages more enzyme is required to achieve optimum performance may appear surprising, given that it is known that intestinal viscosity tends to fall with aging (Petersen et al. 1993). However, finisher rations tend to contain more added fat than starters, and because viscosity affects fat digestion more than any other factor (Carre et al. 1992; Dänicke et al. 1995), it may be the case that a given level of viscosity that is acceptable for a 21-d-old bird is too high for a 35-d-old bird for optimum fat digestion. In addition, work by Choct et al. (1995) and Bedford (1996) indicated that there is a considerable interaction between the intestinal microflora and viscosity, with high viscosity favouring detrimental microbial proliferation and fermentation of nutrients in the jejunum and ileum. The microflora continue to increase in numbers throughout the life of the bird, so it may again be a case of a given viscosity level being tolerable for a young bird but detrimental to an older bird because its microflora are more likely to take advantage of the environment.

Work by Bedford and Classen (1992) demonstrated a clear negative effect of high intestinal viscosity on FCR and gain of the broiler chick. Although this is still undisputed today, the relevance of such data to lower viscosity wheat-based

Table 3. Performance results from experiment 1.

	0–21 d old				22–42 d old		
	FCR	Gain (g)	Intake (g)	Viscosity (mPa · s)	FCR	Gain (g)	Intake (g)
Enzyme (U / kg feed) [a]							
0	1.621	641	1 039	11.99	2.356	1 358	3 190
690	1.582	579	916	7.51	2.146	1 365	2 918
1 380	1.493	634	947	4.49	2.161	1 400	3 021
2 070	1.491	629	937	5.19	2.186	1 394	3 042
2 760	1.474	612	901	4.21	2.139	1 350	2 877
3 450	1.509	612	925	3.41	2.097	1 392	2 908
LSD	0.050	19.4	58.9	3.84	0.159	98	196
Predicted optimum	2 708	2 466	2 462	2 808	2 929	None	3 605
p value of regression	0.000 1	0.000 1	0.000 1	0.000 1	0.007 5	0.787 0	0.002 2

Note: FCR, feed-conversion rate; LSD, least-significant difference.
[a] Level of xylanase activity.

Table 4. Main effects in experiment 2.

	Treatment			Wheat variety		
	Control	Enzyme	P^a	Mercier	Riband	P^a
Gain	493	486	0.450	505	473	**0.019**
FCR	2.04	2.00	0.325	1.94	2.11	**0.008**
Intake	1 009	971	0.416	979	999	0.589
Viscosity	4.7	3.5	**0.004**	4.47	4.00	0.107
Ileal digestibility						
Energy	0.674	0.731	**0.020**	0.700	0.705	0.787
Protein	0.721	0.773	**0.007**	0.748	0.746	0.835
Cysteine	0.482	0.656	**0.043**	0.556	0.656	0.155
Methionine	0.768	0.843	**0.023**	0.793	0.818	0.350
Arginine	0.873	0.900	**0.034**	0.881	0.892	0.320
Threonine	0.658	0.744	**0.001**	0.700	0.702	0.844
Lysine	0.808	0.871	**0.001**	0.836	0.843	0.311

Note: Values are means of 12 birds per treatment, 24 birds per main effect of either enzyme or wheat. FCR, feed-conversion rate.

[a] Wheat × enzyme interaction was not significant for all parameters ($P > 0.45$ in all cases). Values given in bold are significant P values.

diets has been questioned on several occasions. Work that disputes this relationship confounds change in intestinal viscosity with either different wheat samples (Allen et al. 1995) or use of vastly different enzyme preparations (Cowan et al. 1994). Data from the present study show a clear relationship between FCR and intestinal viscosity ($r^2 = 0.945\,4$, $P = 0.000\,1$) and indicate that when all other parameters are held constant, increasing viscosity clearly has a negative effect on performance, even at very low viscosities.

Experiment 2: ileal digestibility study

No wheat variety × enzyme interaction was found for any parameter, so only the main effects are given. The data in Table 4 indicate that the effect of enzyme on ileal digestibility of most amino acids was significant and quite dramatic. The concomitant reduction in viscosity (which was very small) suggests that the two events may well be linked. It is also interesting to note that the improvement in cysteine digestibility through the use of the enzyme exceeds the total cysteine contribution of wheat to the entire ration, demonstrating that the benefit of xylanase addition to a wheat-based diet applies to all ingredients, not just the wheat fraction.

Table 5. Fat digestibility and performance of birds fed diets containing tallow or soybean oil.

	Soy oil	Soy oil + enzyme	Tallow	Tallow + enzyme
Weight (21 d)	681*a*	761*b*	128*c*	665*a*
FCR (0–21 d)	1.392*ab*	1.266*b*	2.449*c*	1.474*a*
Jejunal viscosity (mPa · s)	438	32	311	139
Crude-fat digestibility (%)	82.3*a*	87.3*a*	34.0*b*	51.0*c*

Source: Dänicke et al. (1995). FCR, feed-conversion rate.
a–c, Means within a row followed by a different letter are significantly different ($P < 0.05$).

Fecal digestibilities (not shown) were also determined and found to generally present a similar picture, but the magnitude and significance of the enzyme effect were markedly reduced. Such an observation points to the problems of evaluation of enzyme effects through fecal digestibilities. A review by Bedford (1996) presented the argument that determination of digestibility of any nutrient at the fecal level is fraught with problems in viscous grain-based diets, and the present data support this view. Because of the inevitable microfloral utilization and transformation of amino acids from one form to another, any great emphasis on fecal amino acid digestibilities becomes questionable.

Effects on fat digestibility?

Whereas the present work focused on amino acids and protein digestibility, Dänicke et al. (1995) evaluated the effects of viscosity reduction on digestibility of fat, protein, and energy. Their work clearly showed that the problems of high viscosity are far more apparent for the digestion of highly saturated fatty acids (tallow) than for the digestion of unsaturated fats (soybean oil). The results (Table 5) are quite remarkable: growth was virtually eliminated with the tallow-based rations, whereas the soybean-oil rations supported virtually normal growth rates. The enhancement in growth and fat digestibility due to enzyme supplementation of the soybean oil, although large, was completely overshadowed by the enormous response of the supplemented tallow-based diets. This suggests we may be currently undervaluing the energy value of tallow in comparison with soybean oil when enzymes are used, because our computer matrix values are based on determinations of apparent metabolizable energy (AME) in the absence of enzymes.

Because fat is added to the diet and is not a major component of wheat, these data suggest that enzyme addition promotes better digestion of all nutrients, not simply those encapsulated in the wheat endosperm. Thus, these data support a viscosity-based mechanism, more so than a cell-wall-degradation mechanism.

Value of such data

The use of enzymes in diets containing viscous grains results in more efficient nutrient digestion, as demonstrated in this paper. The full value of this effect can be realized by one of two simple methods:

1. The enzyme can simply be added to the existing diet. This will result in an improvement in FCR, provided the diet is not too luxurious in nutrient density.

2. The diet can be reformulated, and the nutrient specification of wheat can be upgraded to take account of enhanced nutrient availability. Effectively, this means adding 6% onto the AME value of wheat (to a maximum of 13 600 kJ/kg) and 10% onto the crude-protein (CP) and amino acid (AA) contents of wheat (on either a total or digestible basis). The resulting diet is a cheaper formulation because the energy and CP–AA specifications are effectively reduced. All the benefit is tied to the wheat fraction of the diet, as it is the wheat fraction that is responsible for the problematic component (that is, arabinoxylan): hence, the greater the wheat fraction, the greater the enzyme response. For barley, these values are 10% for AME and 15% for CP–AA.

Factors affecting intestinal viscosity

The data presented indicate that intestinal viscosity is an important determinant of a bird's performance and that an effective method to minimize the problems stemming from intestinal viscosity is to use an appropriate viscosity-reducing enzyme. Other factors influencing the absolute viscosity determined at gut level may, however, mitigate the viscosity observed:

- Grain inclusion level — the greater the inclusion level, the greater the intestinal viscosity (the relationship is exponential) (Bedford and Classen 1992);

- Grain genotype and environment in which the grain is grown (Campbell et al. 1989; Rose and Bedford 1995);

- Processing of the diet — pelleting tends to increase intestinal viscosity (MacGee and McCracken 1993); and

- Age of bird — the older the bird, the lower the measured viscosity (Petersen et al. 1993).

Such factors must be taken into consideration when deciding whether to use an enzyme in a particular ration.

Conclusion

Elevated digesta viscosity is detrimental to performance. Whether a particular level of viscosity, such as 6 mPa · s, is tolerable may depend on the fat level and type in the ration and the microfloral numbers and species in the intestine. Absolute values alone are perhaps misleading and need to be considered with these other factors.

Digesta viscosity affects performance by reducing the digestibility of all nutrients, not simply those entrapped in the structure of the cereal grain. Hence, the use of a viscosity-reducing enzyme can have far-reaching effects.

The nutritive value of fats, particularly that of tallow, relative to that of vegetable oils may need to be reevaluated in the presence of enzymes.

The full benefit of enzyme use may be realized by reducing the nutrient density of the diet by tying the enhancement in nutrient digestibility to the wheat fraction of the diet.

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Effect of enzyme supplementation of diets on the physiological function and performance of poultry

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Barley, wheat, rice, and rye contain antinutritive factors, such as β-glucans and arabinoxylans, that interfere with digestion and absorption of nutrients when these cereals are fed to poultry. As a result, feed-conversion efficiency and the rate of growth are reduced and the degree of vent pasting is increased (Friesen et al. 1992; Marquardt et al. 1994). This problem is especially serious in the poultry industry. The supplementation of cereal diets with microbial enzymes is well known to counteract these adverse effects (White et al. 1981).

The poultry industry developed rapidly in China in the 1980s and 1990s. Corn, the major source of feed energy for poultry diets, is scarce in some areas of China, so it is necessary to use barley, wheat, rice, or their milling by-products instead. The inclusion of crude-enzyme preparations in diets containing these cereals has great economic potential.

A positive effect has been reported for barley-based diets supplemented with crude-enzyme preparations containing β-glucanase (Classen et al. 1988). In China, most of the barley is raised near the coast. Barley production has excellent prospects, although production has been limited in recent years (Liu and Han 1994). Rice is another major cereal in China. Rice bran, the milling by-product of rice, is an important, traditional poultry feed. Therefore, one of the objectives of our research was to determine whether enzyme preparations can improve the nutritive value of barley, rice, and rice bran for poultry.

The mechanism by which enzymes improve the nutritive value of cereals was discussed by Francesh et al. (1994). Their assumption was that enzymes that are added to the diet complete the digestive function of the gastrointestinal tract in monogastric animals, thereby improving the digestion and absorption of nutrients. However, animal performance is also closely related to the regulation of metabolism and functioning of the endocrine system. Therefore, our research group had an interest in this problem.

Table 1. Body-weight gain and feed-conversion efficiency in chicks (7–21 d old) fed a barley-based diet supplemented with crude-enzyme preparations.

Experiment	Barley in diet (%)	Crude-enzyme preparation		Body-weight gain (%)	Feed–gain ratio
		Enzyme [a]	(%)		
1	31	CEP	0.025	13.7*	2.37
		CEP	0.2	18.9*	2.28
		Control	0	—	2.55
2	50	CEP	0.025	4.0	2.85
		CEP	0.1	12.5*	2.63
		NEP I	0.1	8.0*	2.68
		NEP I	0.2	9.4*	2.76
		Control	0	—	2.85
3	66	CEP	0.1	6.0	2.16
		NEP I	0.1	2.3	2.23
		Control	0	—	2.28
4	57.4	CEP I	0.01	4.1	2.36
		NEP I	0.1	10.9*	2.17**
		NEP II	0.2	10.8	2.21*
		Control	0	—	2.35
5	57.4	CEP	0.1	6.0	
		NEP I	0.1	8.2	
		Control	0	—	
6	50	CEP	0.1	8.8*	2.36*
		NEP I	0.1	7.0	2.37*
		Control	0	—	2.49

[a] CEP, commercial enzyme preparation; Control, barley-based diet with no added enzymes; NEP, native enzyme preparation.
* ** Significant at $P < 0.05$ and $P < 0.01$, respectively (relative to control diet).

Bioassays in chicks

Bioassays were conducted on male chicks of a laying breed at 7–21 d of age to determine the effect of enzyme preparations on poultry *in vivo*. The results of six trials (Table 1) using barley-based diets containing 31–61% barley and supplemental crude-microbial-enzyme preparations (0.01–0.2%) demonstrated that these diets increased the body-weight (BW) gain of chicks by 2.3–18.9% (average of 10.8%, $P < 0.01$), about the same result as obtained with a corn-based control diet. Feed-conversion efficiency also improved significantly. BW gain was greater with

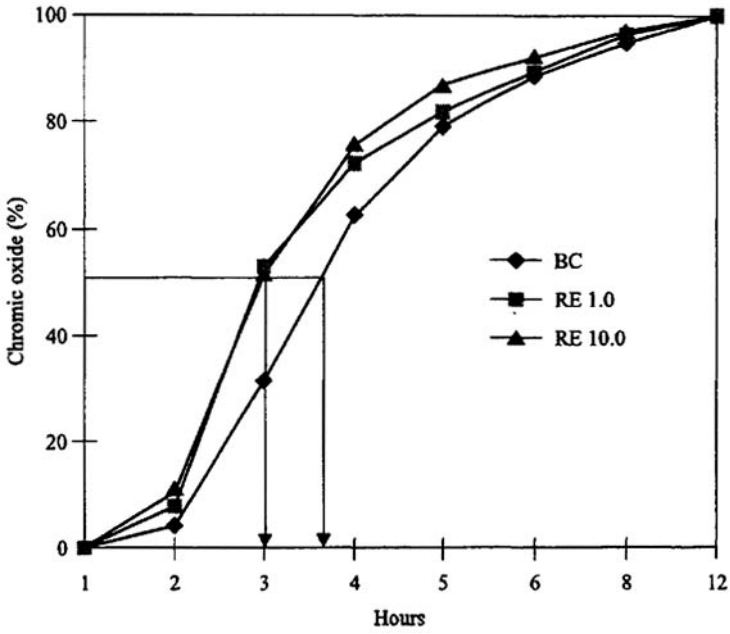

Figure 1. Effect of crude-enzyme supplementation of a barley-based diet (BC) on cumulative Cr_2O_3-excretion curves in 21-d-old chicks: RE 1.0 and 10.0 refer to the amounts of commercial enzyme added to the diet (mg/kg).

0.2% enzyme than with 0.1% enzyme; on the other hand, with an overdose of 1% enzyme, BW gain and feed-conversion efficiency decreased by 6.7 and 6.8%, respectively. Clearly, enzymes added to barley-based diets produce very dramatic improvements in BW gain and feed–gain ratios, provided the concentrations are optimal. Positive results were obtained with both a commercial enzyme preparation (CEP) and a native enzyme preparation (NEP).

Digestive function

In the subsequent studies, corn- and barley-based diets were used as reference diets for investigating the effect of supplemental β-glucanase on some digestive functions of chicks.

Passage rate of digesta through the gastrointestinal tract

Two experiments using the Cr_2O_3 method were carried out on 21-d-old chicks fed barley-based diets (containing 57.4% barley) to estimate the rate of passage of digesta. Figure 1 shows that the time needed for passage of digesta decreased from 3.5 h to 3 h when enzymes were added to the barley-based diet.

Table 2. Digestibility of nutrients in diets.

Diet [a]	DMD [b] (%)	OMD [c] (%)	U-APD [d] (%)	Excreta [e] (%)
BC	73.6±0.7	77.4±0.6	80.3±1.8	26.4±0.7
CC	77.3±1.9*	82.8±1.9**	87.7±2.6**	22.7±1.9**
BC + 0.1% CEP	77.1±1.1**	80.7±1.0**	85.2±5.1*	22.9±1.1**
BC + 1% CEP	57.4±8.0**	62.7±7.0*	72.8±4.8	42.5±7.9**
BC + 0.1% NEP I	75.7±1.1*	79.6±1.2*	83.4±3.0	24.3±1.1*
BC + 1% NEP I	60.5±2.8**	65.5±2.9**	76.2±4.9	39.5±2.8**

[a] BC, barley-based control; CC, corn-based control; CEP, commercial enzyme preparation; NEP, native enzyme preparation.
[b] Dry-matter digestibility.
[c] Organic-matter digestibility.
[d] Apparent protein digestibility.
[e] Percentage of feed intake.
*,** Significant at $P < 0.05$ and $P < 0.01$, respectively (relative to BC).

Digestibility

In chicks fed a barley diet, the digestibility of nutrients was found to be affected by the type and amount of enzyme used in the treatment (Table 2). In general, 0.1% CEP and 0.1% NEP improved dry-matter digestibility (DMD), organic-matter digestibility (OMD), and apparent protein digestibility (U-APD), but the effects were somewhat better with CEP than with NEP. The improvements in DMD, OMD, and U-APD were 4.8, 4.3, and 6.1%, respectively. The digestibilities were also similar to those obtained with the corn control diets. In contrast, 1% enzyme added to the diet greatly decreased DMD, OMD, and U-APD. Therefore, the lower (but not the higher) concentrations of enzymes were highly effective at increasing the digestibility of nutrients in barley-based diets, and these values were similar to those obtained with the corn diet. The reason for the negative effects of the very high concentration of enzymes was not established, but this has also been reported by other researchers.

Viscosity of digesta supernatant in the small intestine

The viscosity of digesta supernatant in the jejunum was found to be significantly higher in chicks fed the barley diet than in those fed the corn diet (2.67 ± 0.37 cP versus 1.87 ± 0.21 cP [1 cP = 0.001 Pa·s], $P < 0.01$). However, the viscosity of digesta in the barley-fed chicks was lowered dramatically (Table 3, $P < 0.05$) when the diet was supplemented with 0.1% crude enzyme. The β-glucan contents of the digesta obtained from the jejunum and ileum increased markedly and were negatively correlated with viscosity ($r^2 = -0.37$ and -0.48, respectively, $P < 0.05$).

Table 3. The β-glucan content and viscosity of a water-soluble extract of digesta from the small intestine of barley-fed chicks.

Diet [a]	Intestine	β-glucan (mg/mL)	Viscosity (cP) [b]
BC	Jejunum	2.93±0.10	2.67±0.37
	Ileum	5.13±1.71	2.79±0.08
BC + 0.1% CEP I	Jejunum	5.85±2.13**	2.24±0.42
	Ileum	22.19±6.37**	2.39±0.21*
BC + 1% CEP I	Jejunum	10.10±4.82	1.95±0.16**
	Ileum	31.79±8.14**	2.52±0.14*

[a] BC, barley-based control; CEP, commercial enzyme preparation.
[b] 1 cP = 0.001 Pa · s.
*,** Significant at $P < 0.05$ and $P < 0.01$, respectively (relative to BC).

This suggests that the β-glucan content in the digesta does not reflect the viscosity of the supernatant *in vivo*. The β-glucan may have been partially hydrolyzed by the crude-enzyme preparations containing glucanase such that the viscosity decreased but the digestibility of the β-glucan was unaffected (Bredford and Classen 1992). The mucin in the digestive juice and dextran from feed starch may also have played a role in the viscosity of digesta from the supernatant.

Disaccharidase activities in the mucosa of the small intestine

Disaccharidase and peptidase are major enzymes in the mucosa of the small intestine. In this experiment, changes of disaccharidase activities in the small intestine of chicks and their effects on digestion were studied in chicks fed barley-based diets containing crude-enzyme preparations. Chicks 7 d old were allocated randomly to four groups and were fed one of the following diets until they were 21 d old: (1) barley-based diet as the control, (2) corn-based diet as normal control, (3) barley-based diet supplemented with 0.1% CEP, and (4) barley-based diet supplemented with 0.2% NEP I.

Both enzyme preparations had much higher cellulase (cellulobiase) than maltase activities (Table 4). However, no cellulase activity was found in the mucosa of the small intestine. Figure 2 shows that the sucrase and maltase activities

Table 4. Disaccharidase activities of two crude-enzyme preparations.

Crude enzyme [a]	Cellulobiase (U/mg)	Lactase (U/mg)	Treholase (U/mg)	Maltase (U/mg)	Sucrase (U/mg)
CEP III	23.98	1.06	2.83	6.33	0.77
NEP I	43.38	0	0.68	16.40	90.87

[a] CEP, commercial enzyme preparation; NEP, native enzyme preparation.

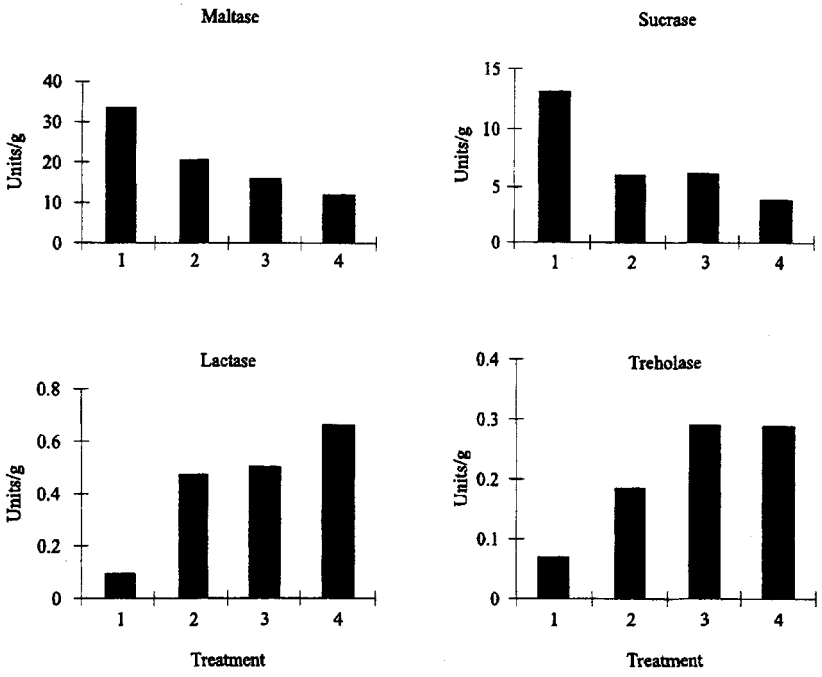

Figure 2. Effect of four dietary treatments on disaccharidase activities in chick jejunum. The treatments were (1) barley control, (2) corn control, (3) barley control + 0.1% enzyme, and (4) barley control + 0.2% enzyme.

were much less with the enzyme-supplemented diets than with the barley control diet ($P < 0.01$). In contrast, the treholase and lactase activities were significantly higher. Previous studies showed that cellulobiase cannot be adsorbed on the intestinal mucosa for digestion. In the current study, sucrase and maltase activities decreased and treholase and lactase activities increased after crude-enzyme preparations were included in the diet. The results suggest that the disaccharidases produced in the intestinal cells may be affected by exogenous enzymes that cannot be adsorbed on the intestinal mucosa and therefore do not participate directly in the digestion of sugars in the membrane. When enzymes are added to barley-based diets fed to chicks, the activities of the four disaccharides are generally similar to those obtained with a corn-based diet.

Pancreatic secretion, activities of pancreatic enzymes, and nucleic acid contents

Mature geese fitted with a permanent pancreatic–duodenal cannula were used to study the effects of exogenous enzymes on the pancreas. The addition of 0.1% of crude-enzyme preparation to a barley-based diet (containing 45% barley) affected

Table 5. Nucleic acid content of the pancreas in chicks (21 d old).

	CC [a]	BC [b]	BC + CEP III [c]	BC + NEP I [d]
RNA (μg/g)	3 926±587*	4 697±497	3 865±687*	4 057±476*
DNA (μg/g)	594±67	601±96	636±80.4	603±68
RNA–DNA ratio	6.61±0.67*	7.60±0.97	5.89±0.65*	6.72±0.86*

[a] Corn-based control diet.
[b] Barley-based control diet.
[c] Barley-based control diet + 0.1% commercial enzyme preparation III.
[d] Barley-based control diet + 0.1% native enzyme preparation I.
* Significant at $P < 0.05$ (relative to BC).

the circadian secretion pattern of several enzymes. Adding enzymes to the diet decreased the overall secretion of the pancreas by 17.7%, lowering it by 43.2% during the day and increasing it by 30.9% at night (constraint of feeding). Activities of protease were elevated by 45.3%: 28.6% ($P < 0.05$) during the day and 83.9% ($P < 0.01$) at night. However, adding enzymes decreased the pancreatic amylase activities by 34.7%, decreasing total activity by 59.8% ($P < 0.01$) during the day and by 21.6% ($P < 0.05$) at night. The overall results suggest that humoral regulation plays an important role in the secretion of digestive enzymes in poultry. Changes in total pancreatic digestive-enzyme activities demonstrated that enzymes added to the diet can affect the activities of endogenous enzymes.

The nucleic acid content in the pancreas was also measured in 21-d-old chicks fed four different diets (Table 5). There was no significant difference in the DNA contents, indicating that the numbers of pancreatic cells among the four groups were similar. However, the RNA content was lower in the enzyme-treated groups ($P < 0.05$) than in the group fed the unsupplemented barley diet. The RNA–DNA ratio was also greater in the barley-based control group than in the groups fed the corn diet and the supplemented barley diets, suggesting hypertrophy of the pancreas in chicks fed the barley-based control diet.

Weight of the digestive organs

Compared with the corn-based diet, the barley-based diet increased the weight of the digestive organs, owing to the antinutritive factors (β-glucans, arabinoxylans) in these feeds. The weight of the digestive organs, however, was reduced after the barley-based diet was supplemented with crude-enzyme preparations. The effects of enzymes on the weight of the gastrointestinal tract were similar to those obtained using a rice-bran diet supplemented with enzymes (Wang et al. 1995).

These data suggest that barley-based diets may increase the output of the pancreatic secretions (Ikegami 1990) and that adding a crude-enzyme preparation

Table 6. Metabolic hormone levels of peripheral blood in chicks (21 d old).

	CC [a]	BC [b]	BC + CEP I [c]	BC + NEP I [d]
TSH (μIU/mL) [e]	1.66±0.45	1.38±0.47	2.24±0.36**	1.85±0.32*
T_3 (ng/mL) [f]	2.95±0.48*	2.35±0.73	3.28±0.64*	3.01±0.54*
T_4 (ng/mL) [g]	12.19±2.95	12.91±1.99	12.28±2.61	12.36±1.89
T_3–T_4 ratio	0.242±0.021*	0.182±0.018	0.267±0.024*	0.244±0.016*
GH (ng/mL) [h]	0.414±0.075	0.405±0.104	0.415±0.075	0.411±0.065
Ins (μU/mL) [i]	12.91±0.55	12.08±1.56	13.50±2.06	12.89±0.88

[a] Corn-based control diet.
[b] Barley-based control diet.
[c] Barley-based control diet + 0.1% commercial enzyme preparation I.
[d] Barley-based control diet + 0.1% native enzyme preparation I.
[e] Thyroid-stimulating hormone.
[f] Tri-iodothyronine.
[g] Tetra-iodothyronine.
[h] Growth hormone.
[i] Insulin.
* ** Significant at $P < 0.05$ and $P < 0.01$ (relative to BC).

containing β-glucanase to the diet can decrease the functional burden in the pancreas caused by viscous carbohydrates in the diet.

Metabolic hormone levels in peripheral blood

Barley-based diets supplemented with crude enzymes also affected the metabolism of poultry. We used radioimmunoassays to measure the concentrations of metabolic hormones in the peripheral blood of chickens, geese, and ducks. Experiments on 21-d-old chicks showed that the concentrations of thyroid-stimulating hormone (TSH) and tri-iodothyronine (T_3) with the enzyme-supplemented barley-based diets were significantly higher ($P < 0.05$) than with the barley-based control diet. Typical results are shown in Table 6. The T_3 concentration in chicks fed the barley-based control diet was approximately 20% ($P < 0.05$) lower than that in chicks fed the corn-based diet; the concentrations of other metabolic hormones, such as TSH, growth hormone (GH), and insulin (Ins), were also lower. It is interesting that the concentrations of TSH in the blood of chicks fed the enzyme-supplemented barley-based diets were higher than those in the blood of chicks fed the corn diet.

Similar results for T_3, GH, and Ins levels were obtained with 42-d-old broilers: the enzyme-treated groups had significantly higher concentrations of these hormones than broilers fed the barley-based control diet (Table 7).

Additional experiments with the 42-d-old broilers showed that the group fed the corn diet had higher concentrations of Ins and lower concentrations of glucagon ($P < 0.05$) than the group fed the barley-based control diet did (Table 8).

Table 7. Metabolic hormone levels in broiler chickens (42 d old).

	CC [a]	BC [b]	BC + CEP I [c]
TSH (μIU/mL) [d]	2.60±0.37	2.24±0.23	2.19±0.30
T_3 (ng/mL) [e]	1.88±0.13	1.81±0.11	2.04±0.23*
T_4 (ng/mL) [f]	12.09±2.69*	14.38±3.61	13.73±4.38
T_3–T_4 ratio	0.149±0.012*	0.126±0.017	0.149±0.013*
GH (ng/mL) [g]	0.752±0.112	0.670±0.097	0.880±0.104*
Ins (μU/mL) [h]	15.51±2.51*	12.52±1.55	13.41±1.70*

[a] Corn-based control diet.
[b] Barley-based control diet.
[c] Barley-based control diet + 0.1% commercial enzyme preparation I.
[d] Thyroid-stimulating hormone.
[e] Tri-iodothyronine.
[f] Tetra-iodothyronine.
[g] Growth hormone.
[h] Insulin.
* Significant at $P < 0.05$ (relative to BC).

Table 8. Blood levels of insulin and glucagon in broiler chickens (42 d old).

	CC [a] ($n = 10$)	BC [b] ($n = 10$)	BC + crude enzymes ($n = 10$)
Ins (μU/mL) [c]	15.5±2.5*	12.5±2.7	13.4±1.6
Glucagon (pg/mL)	168±13*	360±21	304±84
Ins–glucagon ratio	0.09±0.21*	0.037±0.008	0.044±0.009

[a] Corn-based control diet.
[b] Barley-based control diet.
[c] Insulin.
* Significant at $P < 0.05$ (relative to BC).

Adding enzymes to the barley-based diet produced the opposite effect: the enzyme-treated group had higher concentrations of Ins and lower concentrations of glucagon than the barley-based control group. These results suggest that crude enzymes accelerate sugar absorption, affecting the levels of Ins and glucagon and thereby indirectly regulating blood glucose.

Experiments were also carried out with growing geese. Two groups of geese were fed barley-based diets from the time they were 6 d old until they were 60 d old. The first group received unsupplemented barley; the second, barley plus 0.1% crude enzyme. Blood samples were collected from the wing vein of the 60-d-old geese, and blood hormone concentrations were measured. The blood concentrations of TSH, T_3, GH, Ins, and insulin-like growth factor I (IGF-I) were

Table 9. Changes in metabolic hormone levels in growing geese (60 d old) fed a barley-based diet.

	BC [a]	BC + crude enzyme	Difference (%)
TSH (μIU/mL) [b]	1.64±0.32	2.04±0.20	+24*
T_3 (ng/mL) [c]	1.19±0.08	1.39±0.12	+16
T_4 (ng/mL) [d]	10.80±0.75	8.82±1.14	−18*
T_3–T_4 ratio	0.11	0.16	+42
GH (ng/mL) [e]	0.46±0.11	0.63±0.08	+37**
Ins (μU/mL) [f]	4.98±0.37	6.81±0.86	+37**
IGF-I (ng/mL) [g]	44.57±10.00	74.23±16.32	+67**

[a] Barley-based control diet.
[b] Thyroid-stimulating hormone.
[c] Tri-iodothyronine.
[d] Tetra-iodothyronine.
[e] Growth hormone.
[f] Insulin.
[g] Insulin-like growth factor I.
*,** Significant at $P < 0.05$ and $P < 0.01$, respectively (relative to BC).

significantly higher in the enzyme-treated group than in the control group (Table 9).

Experiments with male chicks, broiler chickens, and growing geese showed that adding crude enzymes to the diet increased the blood concentration of T_3 and correspondingly changed the concentrations of tetra-iodothyronine (T_4) and TSH, suggesting that crude enzymes have physiological effects similar to those of thyrotropin-releasing hormone (TRH).

A close relationship between the TRH–TSH–T_3–T_4 axis and GH has been found, and this axis plays an important role in poultry growth (Cogburn et al. 1995). In our experiments the blood levels of GH, IGF-I, and T_3 were elevated simultaneously in diets with added enzymes. The crude-enzyme preparation containing protease may produce a physiologically active peptide when digesting chyme. In addition, the crude-enzyme-microbial preparation may have had some growth-promoting substances that could have affected the cell receptors, thereby producing a physiological effect. The other possibility is that the enzyme preparation enhanced the digestion of feed and the absorption of nutrients, which in turn could have had an indirect effect on hormone concentrations. The results presented in Table 6 show that among the four groups of chicks, the blood concentration of TSH increased in enzyme-treated chicks, whereas that of T_4 did not seem to be affected. However, the concentration of T_3 was obviously elevated. These results suggest that the enzymes directly or indirectly promoted an enhanced activity of deiodinase in liver and kidney tissues, promoting the transformation of T_4 into T_3

(Darras and Vanderpooten 1991). This in turn was responsible for an enhanced rate of metabolism and accelerated growth in poultry. A similar phenomenon (that is, increased thyroid function and rate of growth) has been reported for several species and strains of poultry — including broilers, layers, cockerels, ducks, and geese — when fed barley diets supplemented with crude enzymes.

Immunity

In preliminary studies on 28-d-old chicks, higher concentrations of serum albumin and lower concentrations of serum γ-globulin were found in birds fed barley diets containing 0.1% crude enzyme than in birds fed the same diet without enzyme. In addition to increases in the albumin–γ-globulin ratio ($P < 0.05$), increases were observed in the ratio of spleen or bursa of Fabricius to BW and the number of mature lymphocytes in the enzyme-treated group.

In another study, chicks 7–21 d old were fed a barley-based diet (containing 50% barley) with and without supplemental enzyme (0.1% CEP II). The addition of enzymes to the diet increased BW gain by 4.8% and lowered the feed-conversion rate (FCR) by 3.2% ($P < 0.01$). It also elevated the T-lymphocyte transformation rate by 31.0% ($P < 0.05$); this rate was further increased to 74.2% ($P < 0.01$) at 28 d of age. The T-lymphocyte transformation rate was detected using the ^{3}H-TdR incorporation test. These results indicate that adding crude enzymes to a barley-based diet can enhance cell immunity in chicks. This may be an indirect effect and is probably influenced by the enhanced rate of absorption and utilization of nutrients resulting from the addition of the crude-enzyme preparation. Although the cell immunity increased, no improvements were seen in humoral immunity.

Growth performance

Broiler chickens

Two experiments were carried out on broilers 7–42 d old. One group was fed a barley-based diet (containing 50% barley), and the other was fed the barley-based diet supplemented with 0.1% crude-enzyme preparation. The BW gains of the enzyme-treated birds were 10.4% greater ($P < 0.05$) than those of the birds fed the unsupplemented diet, and they also had better feed-conversion efficiencies. The results of the slaughter test revealed that the proportion of carcass dressing and the proportion of breast muscle (relative to BW) increased by 2.0 and 6.9%, respectively ($P < 0.05$), with enzyme supplementation, whereas the proportion of

Table 10. Productive performance in broiler chickens (7–42 d old) (experiment 2).

	BC [a]	BC + crude enzyme	Difference (%)
Body-weight gain (g)	1 245±123	1 375±89*	+10.4
Feed–gain ratio	2.23	2.15	−3.6
Percentage of body weight			+2.0
Carcass	56.1±1.1	57.2±11.2*	+2.0
Breast muscle	16.8±1.6	17.9±1.1*	+6.9
Thigh muscle	22.1±1.4	21.6±1.0	−2.4
Liver	2.7±0.4	3.2±0.5*	+18.1

[a] Barley-based control diet.
* Significant at $P < 0.05$ (relative to BC).

thigh muscle decreased by 2.4% ($P > 0.05$). In addition, the liver weight–BW ratio was elevated ($P < 0.05$). A summary of the results obtained in experiment 2 is given in Table 10.

The results of both experiments indicate that enzymes added to barley-based diets improved feed-conversion efficiency, accelerated growth, and increased the carcass-dressing percentage. In experiment 2 the increase in breast-muscle-weight–BW ratio and the decrease in the thigh-muscle-weight–BW ratio indicate differential effects in different muscle types. In experiments involving a β-receptor agonist, a similar pattern of response was also observed in ducks (Zhou and Han 1994). The increased liver-muscle-weight–BW ratio and blood T_3 concentrations and decreased uric-acid concentrations indicate enhanced metabolic activity, particularly in protein anabolism, in birds fed the diets with added enzymes.

Geese

Szechuan × White Taihu crossbred growing geese were fed a barley-based diet (45% barley) with or without a crude-enzyme preparation (that is, CEP) from the time they were 7 d old until they were 60 d old. The BW gain was 18.5% higher ($P < 0.01$, Figure 3) and the FCR 10.2% lower in the enzyme-treated group. The rate of growth for the two groups was significantly different ($P < 0.05$) at 7–35 d of age but was not quite different at 35–60 d of age (Figure 4). However, the BW of the enzyme-treated group was still higher than that of the control by 13.3% ($P < 0.01$). These results suggest that the more mature geese did not respond as well to enzyme treatment as the younger geese, which may be related to the more highly developed gastrointestinal tract of the mature bird. Similar results have been observed in chickens.

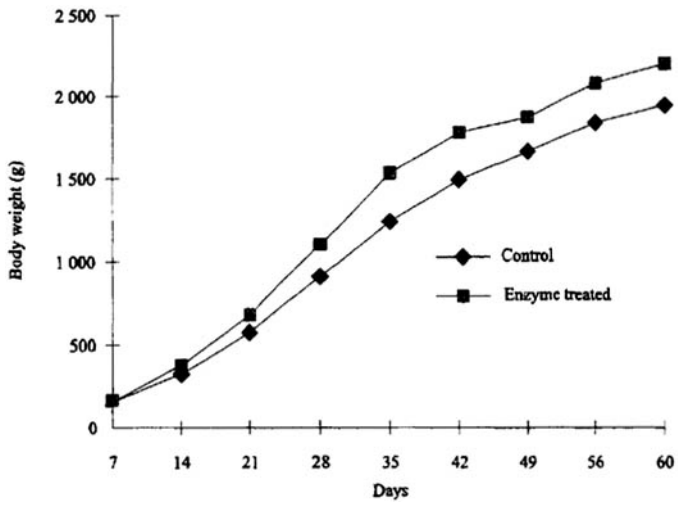

Figure 3. Effect of crude-enzyme supplementation of a barley-based diet on the weight of growing geese at 7–60 d of age. The dietary treatments were the barley control and the barley control + 0.1% enzyme. Weight on the *Y* axis represents the average weight of the geese.

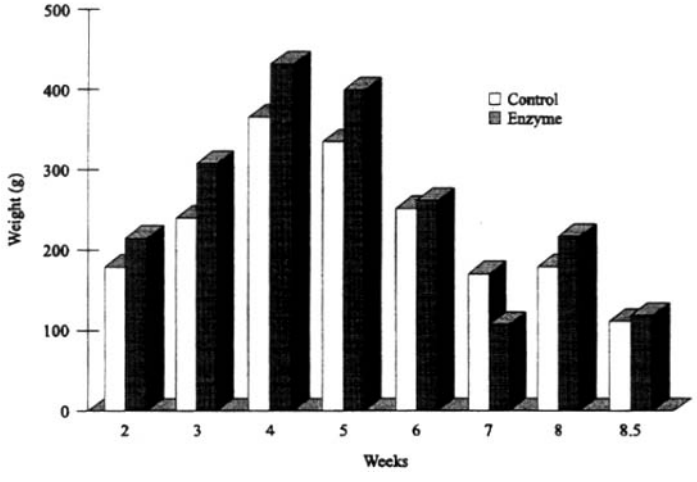

Figure 4. Effect of crude-enzyme supplementation on the body-weight gain in growing geese. Data taken from Fig. 3.

Meat ducks

Two experiments were conducted on meat ducks (Cherry Valley). In experiment 1, 0.1% crude-enzyme preparation (that is, CEP) was added to a diet containing 60% barley. The BW gain of the 20-d-old ducks fed the enzyme-treated diet was

15.4% higher ($P < 0.01$) than that of the ducks in the control group, which received no enzyme treatment. Thereafter, the growth rate slowed, and the difference between the two groups was not significant at 42 d of age. In experiment 2, the BW gain of ducks 21 d old increased by 18.6% (426 ± 62 g versus 359 ± 24 g, $P < 0.05$) when the diet was supplemented with enzymes. At 42 d of age, the difference was 20.1% (490 ± 118 g versus 408 ± 52 g, $P < 0.05$). The barley diet in experiment 2 was at a lower plane of nutrition than that in experiment 1. The results of these studies suggest that the response of ducks to enzyme treatments is affected not only by the age of the ducks but also by the plane of nutrition.

Effects of crude enzymes in diets containing rice bran

Male chicks 7–21 d old were used to study the effects on chick performance of enzymes added to rice-bran diets. BW gain and feed-conversion efficiency were improved when 0.5% crude enzyme was added to a diet containing 40% rice bran, but the differences were not significant ($P > 0.05$). However, beneficial effects of enzymes (0.2 and 0.5%) were observed, especially for the chicks 7–14 d old that were fed 25% rice bran (Table 11). These results support the conclusions of Wang et al. (1995), who found that enzymes can improve the nutritional value of rice bran added to the diet of chickens and that the response is influenced by the concentration of rice bran in the diet.

Table 11. Body-weight gain and feed-conversion efficiency in chicks (7–14 d old) fed rice-bran diets (25% rice bran) supplemented with crude-enzyme preparations.

Crude-enzyme preparation [a]		Body-weight gain [b]	Feed–gain ratio [b]
Enzyme	(%)	(%)	(%)
EE	0.2	11.80**	−2.57
	0.5	8.80	−8.14**
ZW	0.2	18.91*	−8.73*
	0.5	18.94**	−9.62*
CEP	0.2	7.56	−3.34
	0.5	14.39**	−2.14

[a] EE and ZW are enzyme products; CEP, commercial enzyme preparation (same as the one used in the barley-diet experiments.)

[b] Percentage improvement in performance of chicks fed the rice-bran + enzyme diet, compared with the performace of chicks fed the rice-bran control diet (with no added enzymes).

*,** Significant at $P < 0.01$ and $P < 0.05$, respectively (relative to rice-bran-based control diet).

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Enzymes in animal nutrition: the unseen benefits

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Nonstarch polysaccharides (NSPs) in monogastric diets have an antinutritive activity, which is manifested by wet droppings and poor use of nutrients. Microbial enzymes targeting these polymers have, for this reason, shown highly positive results in both enhancement of performance and reduction of excreta volume and moisture. A decreased output of organic matter (OM) to the environment is an important attribute of feed enzymes because excessive output of nitrogen and phosphorus is a major problem in densely populated areas.

Enzymes also allow the use of a wide range of ingredients without compromising bird performance and hence provide great flexibility in least-cost feed formulation. Alleviation of gastrointestinal morbidity and other diseases in animals, such as swine dysentery and acidosis in ruminants and horses, is another possible benefit of feed enzymes.

Increased nutrient digestibilities and decreased excreta output

Dry-matter digestibility (DMD) in animals ranges from 50 to 80%; the remainder of the dry matter (DM) is lost via the excreta. In the poultry industry, for instance, this represents 9 000–22 000 t of high-N manure per million birds annually. In densely populated parts of the world, such as Asia and Europe, excretion of large amounts of OM, especially OM containing high levels of nitrogen and phosphorus, presents serious environmental problems. In recent years, enzymes have been widely used in monogastric diets to increase nutrient digestibilities and to decrease nutrient waste in the excreta. The effect of enzyme supplementation on DMD in pigs and poultry depends on the type of diet and the type of animal: increases in DMD range from 0.9 (Schutte et al. 1995) to 17% (Annison and Choct 1993) in poultry and from 0 (Taverner and Campbell 1988) to 5.2% (Schmitz 1995) in pigs.

We compared the efficacy of a glycanase product in a wheat known to have low metabolizable energy and a normal wheat in broiler diets. Some of the results are shown in Table 1. The enzyme supplementation improved DMD by

17%, apparent metabolizable energy (AME) by 24%, and feed-conversion rate (FCR) by 31%, which coincided with a 50% reduction in digesta viscosity.

The enzymes currently used in monogastric diets are predominantly glycanases, which cleave NSPs into smaller polymers, thereby removing their ability to form viscous digesta and enhancing nutrient digestibilities. The effects of glycanases are generally nonspecific, except for their effect on fat (greater effect on saturated fat than on unsaturated fat). Another enzyme used in feed is phytase, which increases the utilization of phytate phosphorus. The ability of phytase to improve the digestion of phytate phosphorus and subsequently to reduce the output of organic phosphorus to the environment has attracted a great deal of scientific and commercial interest. Supplementing pig diets with 500 U phytase activity / kg feed increased the digestibility of phosphorus from 44.2% to 52.4% (18.6% increase) and that of calcium from 44.2% to 51.7% (17.0% increase). Supplementing piglet diets with 1 500 U phytase activity / kg feed also significantly improved the weight gain (from 424 g/d to 529 g/d) and FCR (from 1.65 to 1.52) (Jongbloed et al. 1993). In poultry, phytase use was reported to reduce phosphorus excretion by as much as 40% for broilers (Simons and Versteegh 1991). When phytase was added to layer diets, increased egg production and positive effects on egg weight and tibia ash were also noted (Simons and Versteegh 1993).

Cereal by-products, such as rice bran, are important feed ingredients in Asia, but their efficient use in monogastric diets is hindered by the presence of high levels of NSPs and phytate. Martin (1995) demonstrated that supplementing duck diets with microbial phytase allowed rice bran to be used at high levels (up to 60%) without detrimental effects. Phosphorus excretion was reduced by 9.6%, and significant decreases in excretion of manganese, copper, and zinc were also noted.

Table 1. Effect of enzymes on DMD, AME, FCR, and digesta viscosity in broilers fed low-ME and normal wheat diets (n = 8).

Diet	DMD (%)	AME (MJ / kg DM)	FCR	Viscosity (Mpa · s)
Maize control	81.0	16.6	1.96	3.2
Low-ME wheat	65.2	12.0	2.69	20.3
Low-ME wheat + enzymes	76.3	14.9	2.05	10.4
Normal wheat	74.9	14.5	2.01	9.7
Normal wheat + enzymes	76.6	14.8	1.95	5.7

Source: Annison and Choct (1993).

Note: AME, apparent metabolizable energy; DM, dry matter; DMD, dry-matter digestibility; FCR, feed-conversion rate; ME, metabolizable energy.

Reduced excreta moisture

Wet excreta is a big problem in the poultry industry, especially in the case of laying hens, where increased percentages of dirty eggs are associated with wet droppings. In many countries, dirty eggs are unsuitable for sale as second-grade eggs and therefore represent a substantial net loss for the industry. Wet droppings may increase the production of gases (that is, ammonia and hydrogen sulfide) and fly and rodent populations in pig and poultry sheds. These can affect the well-being of the animals by increasing stress and lowering air quality, and they can affect the health of the staff who work in the shed (Donham 1995).

A reduction in the moisture content of poultry excreta is often noted when glycanases are included in the diet. In a recent trial, when we added an equivalent of 4% soluble NSPs to a sorghum-based broiler diet, bird performance was significantly depressed and the excreta moisture increased from 47.4% (in birds fed the basal diet) to 64.5%. Supplementing the NSP-enriched diet with three different commercial glycanase products improved performance, but their effectiveness in reducing the moisture levels of the excreta differed from 10 to 29%. This supports the view that different glycanases have similar performance-enhancement effects in monogastric animals but the site of the breakdown of the NSPs in the gut and the molecular sizes of the released products differ. These important differences determine the efficacy of an enzyme in reducing excreta moisture. Overdepolymerization of NSPs may yield high amounts of osmotically active oligomers in the gut, which in turn increase the moisture content of the excreta (Choct and Annison 1992). Brufau et al. (1993) examined the effects of adding enzymes to broiler diets containing different varieties of barley and reported reductions of as much as 50% in the incidence of sticking and watery droppings. The severity of diarrhea in young pigs fed barley-based diets can also be largely overcome by the use of ß-glucanase (Inborr and Ogle 1988).

The use of enzymes to alleviate the problem of wet droppings is not limited to just pigs and poultry. Millions of household pets create a big environmental problem by dumping sloppy excreta in parklands and residential areas throughout the world, and enzymes have shown great potential for ameliorating this situation.

Improved animal health

Some disease conditions, such as swine dysentery and acidosis in ruminants and horses, have great economic significance and are closely related to the status of the gut microflora (fermentation). For example, swine dysentery is caused by a spirochaete bacterium that resides in the large intestine, and the proliferation of this bacterium is inhibited by low rates of fermentation, as indicated by Siba et

al. (1993, 1995). They showed that diets based on cooked rice and animal proteins can give a high level of protection against swine dysentery. Steam-flaking of maize and sorghum can also eliminate or largely reduce the incidence of swine dysentery, but this type of processing is ineffective for wheat, barley, or groats (Siba et al. 1995). The increased fermentation in the large intestine may be due to an elevated level of NSPs in the diet. In the case of maize and sorghum, steam-flaking may have disrupted the cell-wall matrix, making the starch granules more susceptible to amylase and thereby speeding up starch digestion in the upper part of the gut. Glycanases may offer an alternative solution to the problem. For instance, the production of short-chain fatty acids in the cecum, colon, and rectum was significantly reduced by adding ß-glucanase to a barley-based pig diet (Inborr et al. 1995).

In ruminants, absorption of glucose from the intestine can markedly improve fat synthesis and glycogen stores. The fermentation of starch in the gut can lead to significant clinical and subclinical problems in both ruminants and horses. Secondary problems include laminitis (lameness) and poor utilization of minerals. These problems significantly affect animal welfare, productivity, and waste management (Murray et al. 1991; Godfrey et al. 1992). The nature and quantity of the starch and fibre components influence the extent of digestion or fermentation, as well as the location within the gut where the digestion or fermentation occurs. Although the use of enzymes for ruminants and horses is not widespread, the manipulation of carbohydrate digestion in these species may be a great "hidden benefit" for enzyme users in the future.

The significance of gut microflora to the nutrition of chickens is not well documented. Excessive fermentation in the small intestine may interfere with the normal physiological process of nutrient digestion. As often noted, adding antibiotics to poultry diets that have highly soluble NSPs markedly improved bird performance (Misir and Marquardt 1978). Elevated levels of intact soluble NSPs detrimentally increased the activity of fermentative microorganisms in the small intestine (Choct et al. 1996). Xylanase supplementation largely eliminated fermentation in the small intestine and improved the performance of the birds. A sudden change in the gut ecology (from an aerobic or facultative anaerobic environment to a strictly anaerobic one) may induce gastrointestinal stress and severely affect the normal physiological processes.

Morgan and Bedford (1995) reported that coccidiosis problems could be prevented by using enzymes. Birds fed a wheat-based diet with and without glycanase supplementation showed vastly different responses to coccidiosis challenge. Growth was depressed by 52.5% in the control group but by only 30.5% in the enzyme group, which also had a much better lesion score. An increase in digesta

passage rate and a reduction in excreta moisture are often noted when glycanases are added to poultry diets, which may be detrimental to the life cycle of the organism.

Increased precision and flexibility in least-cost feed formulation

The nutritive value of cereal grains for poultry varies greatly, and no suitable assays are currently available for rapid in-mill testing. For instance, the variability in the AME of wheat for poultry can be as great as 4 MJ / kg DM (Sibbald and Slinger 1962; Rogel et al. 1987). This problem can be largely overcome by using glycanases to bring the AME of different wheats to comparable levels (Choct et al. 1995). In a recent trial, enzyme supplementation increased the AME of a wheat from 13.7 MJ / kg DM to 14.5 MJ / kg DM and reduced between-bird variation by 74% (M. Choct et al., unpublished). Significant reductions in the coefficient of variation in performance of birds fed barley diets have also been reported (Classen et al. 1988). The practical significance of this is that it allows increased precision in least-cost feed formulation, resulting in more uniform poultry performance. Enzymes also allow a wide range of ingredients to be used in a diet for a desired outcome. This gives the producer a great deal of flexibility in formulating a nutritionally balanced, least-cost diet.

Conclusion

Enzymes have changed the way nutritionists select ingredients for a nutritionally balanced, least-cost diet. Using feed enzymes can also alleviate the problem of environmental pollution and control certain diseases. Enzymes will play an indispensable role in 21st-century animal production.

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Practical experience with the use of enzymes

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The use of enzymes has been common in many industries for many years. Traditional applications in the food-processing, brewing, baking, and leather-working industries have been well documented. More recently, the detergent industry began to incorporate specific proteolytic enzymes in detergent preparations (Walsh et al. 1993), and an increased understanding of the properties of enzymes and their function has led to their introduction in the animal-feed industry.

Although the application of enzyme technology is relatively new for the feed industry, the evidence to date suggests that this technology has a future, particularly for poultry and to a lesser extent for swine (Classen and Bedford 1991).

Enzymes have been approved for use in poultry feed because they are natural products of fermentation and therefore pose no threat to the animal or the consumer. Their use in poultry feeds has predominantly been related to the hydrolysis of fibre or nonstarch polysaccharide (NSP) fractions in cereal grains. These NSPs cannot be digested by the endogenous enzymes of poultry and can have antinutritive effects. The two main NSPs in cereals are β-glucan in barley and oats and pentosans in wheat, triticale, and rye (Friesen et al. 1992). Numerous researchers have demonstrated that the soluble-NSP fraction, not the total NSP fraction, is responsible for antinutritive responses (Classen and Bedford 1991). These NSPs can bind to large amounts of water, and as a result, the viscosity of fluids in the digestive tract is increased. The increased viscosity causes problems in the small intestine because it reduces the substrate–enzyme interaction, which reduces nutrient availability (particularly fat) (Friesen et al. 1992) and results in increased amounts of sticky droppings (Boros et al. 1995).

The main goals of enzyme supplementation of poultry diets are to remove or destroy the antinutritive factors in cereals; to enhance the overall digestibility of the feed; to render certain nutrients biologically more available; and to reduce pollution from animal excreta by reducing dry-matter excretion. To achieve these goals, enzymes must be able to survive the processing of feeds and resist the acidic conditions and proteolytic enzymes in the proventriculus and gizzard. Under

Table 1. Average 42-d BW, days to 1 800-g BW, feed-conversion efficiency, and incidence of mortality for broilers fed diets based on wheat–corn, barley, or barley and enzyme.

	Wheat–corn	Barley	Barley + enzyme
Avg. 42-d BW (g)	1 996*a*	1 718*c*	1 891*b*
Days to 1 800-g BW	39.1*c*	43.2*a*	40.6*b*
Feed–gain ratio			
0–42 d	1.75*b*	2.05*a*	1.99*a*
1 800 g BW	1.71*c*	2.08*a*	1.96*b*
Culls + mortality (%)	9.4	9.1	5.7
Feed cost to 1 800-g BW ($)	0.81*a*	o.73*b*	0.70*c*

Source: Campbell et al. (1984).
Note: Avg. BW, average body weight.
a–c, Means within a row followed by the same letter are not significantly different ($P < 0.05$).

practical feedmill conditions, the steam used during pelleting is responsible for lost enzyme activities. Enzymes can be quite stable under a dry heat of 90°C for 30 min if moisture is added in the form of steam at 95°C for 15 min, but almost 80% of the activity is lost (Inborr and Bedford 1994). However, bird performance may be unaffected if feeds are conditioned before pelleting at temperatures below 85°C. Spring et al. (1996) reported that cellulase, fungal amylase, and pentosanase in feeds could be pelleted at temperatures as high as at least 80°C (measured at die outlet) without considerable loss of activity; at extrusion temperatures of 100–120°C the enzyme activity was greatly reduced, although the xylanase activity appeared to be more heat sensitive than the β-glucanase activity (Vukić Vranješ et al. 1994). These data suggest that a normal pelleting temperature of 75–80°C can be used for enzyme-supplemented diets but that at higher temperatures it may be prudent to spray liquid enzyme on the final feed product after conditioning and processing.

In practical poultry feeding, the choice of appropriate enzymes for a particular diet is important. The main enzyme preparations currently available are targeted for barley–soybean-based poultry feeds, corn–wheat–soybean-based poultry feeds, and wheat–rye–soybean-based poultry feeds. Animal nutritionists must therefore be careful to choose the appropriate enzyme preparation for a particular feed. Commercial enzyme preparations (CEPs) are generally species-specific (Ph and temperature) multienzyme cocktails and therefore should be selected on the basis of the manufacturer's recommendations for the species (poultry versus pig) and substrate (barley and oats versus rye, triticale, and wheat).

Adding enzymes to practical cereal-based diets

Campbell et al. (1984) evaluated the practical and economic benefits of supplementing a barley-based diet with a crude-enzyme preparation with a high level of β-glucanase activity. Three diets were compared: a standard wheat–corn diet; a barley-based diet; and a barley-based diet plus enzyme. The study (Table 1) demonstrated that birds fed the barley-plus-enzyme diet scored significantly better on performance parameters than those fed the barley diet without enzyme. Although the performance obtained with the barley-plus-enzyme diet did not match the level of that obtained with the wheat–corn diet, it was clearly demonstrated that given the right economic conditions (that is, barley prices lower than wheat and corn prices) the feed cost per bird could be greatly reduced using the alternative grain-plus-enzyme diet.

Based on the above study, nutritionists were looking for additional ways to reduce feed in broiler diets. To find out whether enzyme supplementation was necessary only during the first few weeks of the bird's life, a study using broiler chicks was conducted to compare the effects of barley diets with and without enzyme supplementation and those of a standard commercial wheat diet (Rotter et al. 1989). The barley-plus-enzyme diet was fed for 2, 4, or 6 weeks. Although substituting barley for wheat in broiler diets had little effect on the feed consumption of birds, very significant results were obtained for body-weight (BW) gain, feed-conversion efficiency, and vent pasting (Table 2). The barley used in this study was a hull-less barley, high in soluble β-glucan, and feeding it resulted in a high incidence of sticky droppings, as indicated by the incidence of pasted vents at 7 d of age. The enzymes in the diet significantly reduced the incidence of sticky

Table 2. Effect of feeding enzyme-supplemented hull-less barley (Scout) on feed consumption, BW gain, feed–gain ratio, and vent pasting of broiler chicks (6 weeks).

Treatment	Feed consumption (g)	BW gain (g)	Feed–gain ratio	Vent pasting (%)
Wheat	2 910*b*	1 529*bc*	1.90*b*	10.0*c*
BC [a]	2 941*ab*	1 473*c*	2.00*a*	73.8*a*
BC + Enz (for 2 weeks)	2 978*ab*	1 539*bc*	1.93*ab*	21.4*b*
BC + Enz (for 4 weeks)	3 047*a*	1 598*ab*	1.91*b*	19.4*b*
BC + Enz (for 6 weeks)	2 992*ab*	1 638*a*	1.83*c*	18.9*b*
SE	29.8	21.3	0.02	1.4

Source: Rotter et al. (1989).

Note: BC, barley control; BW, body weight; Enz, enzyme (crude cellulase [Celluclast], Novo A/S Denmark); SE, standard error.

[a] Starter diet: 56.4% barley; grower diet: 67.8% barley.

a–c, Means within a column followed by the same letter are not significantly different ($P < 0.05$).

Table 3. Practical application of three CEPs in wheat-based broiler diets fed to male broilers.

		Control diet +		
	Control	Enz-1	Enz-2	Enz-3
Avg. 42-d BW (g)	2 256*b*	2 283*ab*	2 313*a*	2 328*a*
SD	40	30	29	23
CV	0.018	0.013	0.013	0.010
Avg. feed consumption (g/bird)	4 303	4 309	4 322	4 170
Feed–gain ratio	1.94*a*	1.92*a*	1.90*a*	1.82b
Litter moisture, week 6 (%)	40	43	40	40

Source: From study 1, 1994, University of Manitoba.

Note: Avg. BW, average body weight; CEP, commercial enzyme preparation; CV, coefficient of variation; Enz-1, -2, and -3, enzyme preparations 1, 2, and 3; SD, standard deviation.

a,b, Means within a row not followed by the same letters are significantly different ($P < 0.05$).

droppings. The data also clearly indicated that continuous feeding of enzymes resulted in performance superior to that of the wheat control group. The authors concluded that to maximize the benefits of enzyme supplementation, the enzyme diet must be fed to the birds to market age. More recent practical experience has confirmed these findings.

Comparative enzyme studies

One of the problems encountered by feed-industry nutritionists is choosing enzyme preparations appropriate to their particular situation. Numerous companies approach the feed industry in hopes of selling their products. To help a few of the feed mills determine which enzyme preparation is most effective, several studies were conducted in cooperation with the industry. In a series of three large practical studies, several enzyme preparations for wheat- and wheat–barley-based diets were compared. Study 1 involved male broiler chickens fed a wheat-based control diet supplemented with three CEPs at levels recommended by the manufacturer. Although all three enzyme preparations significantly increased BW in comparison with the control, only enzyme preparation 3 (Enz-3) significantly improved feed-conversion efficiency (Table 3). Also of commercial importance is the fact that enzyme supplementation reduced the variability in BW, resulting in a more uniform flock, which the processor prefers.

Study 2 involved a mixed flock (males and females) fed a wheat–barley-based diet supplemented with three enzyme preparations. Enzyme preparation 1 (Enz-1) in study 2 was the same as Enz-3 in study 1; the other two preparations

Table 4. Practical application of three CEPs in wheat–barley-based diets fed to broilers in a mixed-sex group.

		Control diet +		
	Control	Enz-1	Enz-2	Enz-3
Avg. 42-d BW (kg)	1.96*b*	2.03*a*	2.01*ab*	1.98*b*
Avg. feed consumption (kg/bird)	3.86	3.88	3.82	3.79
Feed–gain ratio	2.00*a*	1.94*b*	1.93*b*	1.94*b*
Mortality	3.8	4.0	2.9	3.1
3-week gut viscosity (Cp) [a]	6.1*a*	3.1*b*	3.6*b*	3.5*b*
3-week litter condition (% DM)	68*b*	71*ab*	74*a*	72*ab*
6-week BW variation	0.19	0.17	0.18	0.19

Source: From study 2, 1995, University of Manitoba.

Note: Avg. BW, average body weight; CEP, commercial enzyme preparation; DM, dry matter; Enz-1, -2, and -3, enzyme preparations 1, 2, and 3.

[a] 1 P = 0.1 Pa · s.

a,b, Means within a row not followed by the same letter are significantly different ($P < 0.05$).

were different. Contrary to the findings of study 1, only Enz-1 resulted in significant growth-rate response, whereas all enzyme preparations equally improved the feed–gain ratio (Table 4). As demonstrated by Bedford (1995), reduction in gut viscosity is a major factor contributing to the enzyme response. Data in Table 4 demonstrate that gut viscosity was lowest for Enz-1, suggesting that this preparation was most effective in hydrolyzing the antinutritive NSPs in the diet. Although the results were not significant, the Enz-1 preparation also resulted in the best flock uniformity. All three enzyme preparations tended to reduce litter moisture, thereby creating a drier environment in the poultry house.

Study 3 involved a mixed flock of broilers fed a wheat–barley diet supplemented with four enzyme preparations. Because it was demonstrated in both of the previous studies that enzyme supplementation improved growth or feed-conversion efficiency, or both, no control diet was fed. The four enzyme preparations resulted in equal growth; however, enzyme preparation 4 (Enz-4) significantly lowered feed consumption compared with enzyme preparation 2 (Enz-2); Enz-4 therefore resulted in a significantly better feed–gain ratio than found with Enz-2 or Enz-1 and tended to result in a better feed–gain ratio than Enz-3 did (Table 5). The feed–gain ratios correlated well with the gut-viscosity measure, demonstrating the effectiveness of the preparations. Based on current feed and enzyme prices, the most cost-effective enzyme preparation (the lowest feed cost per kilogram weight) was Enz-4 (the same preparation as Enz-3, study 1; Enz-1, study 2).

Table 5. A commercial study comparing four CEPs in wheat–barley-based diets.

	Wheat–barley diet +			
	Enz-1	Enz-2	Enz-3	Enz-4
Avg 42-d BW (kg)	2.23	2.24	2.23	2.25
Avg. feed consumption (kg/bird)	3.87*ab*	3.91*a*	3.82*ab*	3.78*b*
Feed–gain ratio	1.767*a*	1.770*a*	1.741*ab*	1.703*b*
Mortality (%)	5.0	6.4	7.8	9.3
Gut viscosity (cP) [a]	8.59*a*	5.10*b*	4.93*b*	4.41*b*
Feed cost per kilogram weight ($)	0.482*ab*	0.492*a*	0.478*ab*	0.471*b*

Source: From study 3, 1994, University of Manitoba.
Note: Avg. BW, average body weight; CEP, commercial enzyme preparation.
[a] 1 P = 0.1 Pa · s.
a,b, Means within a row not followed by the same letter are significantly different ($P < 0.05$).

These three commercial-type studies, using pelleted diets prepared under commercial pelleting conditions, demonstrate that not all CEPs result in equal performance, so customers should determine which preparation is most cost-effective under their circumstances.

Upgrading the nutritive value of a cereal

The feed industry formulates poultry diets on the basis of least cost. Under these circumstances, the price of the cereal and the nutrient content will determine whether a cereal enters the computer formulation. With the introduction of enzymes, nutritionists should be able to release more of the nutrients in a particular cereal, thereby making it more competitive with corn. It has been suggested that supplementation with enzymes could increase the energy available in wheat by 6–8% and would result in growth rates similar to, and feed–gain ratios better than, those obtained with a control wheat diet. To investigate this suggestion, a study was conducted using a wheat-based diet formulated without enzymes, based on National Research Council (NRC 1994) nutrient requirements. The same diet was reformulated to upgrade metabolizable energy by 6% and the amino acid content by 10%. This diet was fed with and without enzyme supplementation. The results of this study are shown in Table 6.

The data clearly demonstrate that when the nutrient matrix for wheat is upgraded without enzyme supplementation, the performance of the birds is depressed. However, the same diet supplemented with enzymes yields performance values equal to those of birds fed the control diet, suggesting that upgrading the nutrient content of the cereal will make the cereal cost-effective enough to enter the least-cost formulation.

Table 6. Evaluation of male-broiler performance when nutrient matrix is upgraded in an enzyme-supplemented wheat-based diet.

		Upgraded nutrients [a]	
	Control	Control	Enzyme
Avg. BW (g)			
21 d	750*a*	707*b*	745*a*
42 d	2 256	2 184	2 252
Avg. feed consumption (g/bird)			
0–21 d	1 064	1 037	1 054
0–42 d	4 303	4 237	4 265
Feed–gain ratio			
0–21 d	1.518*b*	1.574*a*	1.513*b*
0–42 d	1.941	1.973	1.922

Source: From 1994 study, University of Manitoba.
Note: Avg. BW, average body weight.
[a] Nutrient values of wheat were upgraded by 6% for nitrogen-corrected apparent metabolizable energy and by 10% for amino acids.
[b] Enzyme used was Avizyme TX @ 1 kg/t.
a,b, Means within a row not followed by the same letters are significantly different ($P \leq 0.05$).

Practical use of rye in broiler diets

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 (arabinoxylans) on the utilization of rye-based diets can be overcome to a considerable degree by adding crude-enzyme preparations with high endo-xylanase activity (Campbell and Bedford 1992). In battery studies, under ideal environmental conditions, the performance obtained in broilers fed an enzyme-supplemented rye-based diet was equivalent to that obtained in broilers fed the control wheat-based diet (Marquardt et al. 1994). These promising results encouraged the authors to determine whether similar growth performance could be achieved under commercial feeding conditions. Preliminary studies had shown that 18% rye in the starter diet and 20% rye in the grower–finisher diet supplemented with an enzyme resulted in BW similar to, but feed–gain ratio lower than, the wheat–barley control diet (Boros et al. 1995). A commercial field study was conducted using a starter diet with 15% rye and a grower–finisher diet with 30% rye replacing barley in a wheat–barley-based control diet. All diets were supplemented with a crude-enzyme preparation, steam pelleted, and fed to 16 000 birds (7 000 males and 9 000 females) each. The rye in the starter diet was included to

Table 7. Comparative performance obtained during a field study with broilers fed a standard barley–wheat- or barley–wheat–rye-based diet supplemented with enzymes.

	Barley–wheat + enzyme	Barley–wheat–rye + enzyme
Avg. 35-d LW (kg)	1.73*a*	1.62*b*
Feed consumption (kg/bird)	3.29	3.47
Feed–gain ratio	1.87*b*	2.10*a*
Frequency of vent pasting (%)	3.06*b*	9.72*a*
Litter condition (scale 1–3) [a]	1.5	3.0
Litter moisture (%)	27.9*a*	39.7*b*

Source: Modified from Boros et al. (1995).
Note: Avg. LW, average live weight.
[a] 1 = normal, 2 = wet, 3 = caked.
a,b, Means within a row not followed by the same letter are significantly different ($P < 0.05$).

reduce the degree of vent pasting and the resulting high litter-moisture content and to allow time for the chicks to adapt to the rye diet.

The final average BW at 35 d of age was 1.62 kg for birds fed the rye diets versus 1.75 kg for birds fed the standard wheat–barley diet, a difference of 7% (Table 7). Other values for broilers fed the rye and standard diets were 2.10 and 1.87 for feed-conversion efficiency (a 12% depression), 9.7 and 3.1% for frequency of vent pasting, 3.0 and 1.5 for litter-condition score, and 39.7 and 27.9% for litter-moisture content, respectively.

The problem of wet litter was very pronounced for broiler chicks fed the rye diets. This was attributed to the higher moisture content and slower rate of evaporation from excreta of rye-fed broilers. The wet litter probably reduced chick performance by increasing the incidence of soiled plumage, resulting in an increased expenditure of feed energy to maintain body temperature. Wet, sticky excreta in rye-fed birds was greater than expected and might be attributable to incomplete hydrolysis of the arabinoxylans, as discussed by Boros et al. (1995).

These results suggest that rye will not be widely used in the feed industry, even if the diet is supplemented with an enzyme preparation high in xylanase activity. Further research should focus on the reduction of the water-binding capacity of excreta from rye-fed birds to improve the environmental conditions for chicks fed rye diets.

Conclusion

1. Enzyme supplementation has been shown to improve the productive value of feeds and to allow the use of more novel feedstuffs without loss of performance.

2. Although all tested enzyme preparations resulted in some improvement in performance (growth or feed–gain ratio, or both), differences do exist in magnitude of the response.

3. When enzymes are used with wheat- and barley-based diets, the enzymes can, on average, improve the nutrient availability by 5 and 10%, respectively.

4. Some problems encountered with rye diets have not been overcome with the use of enzyme preparations currently available.

Considerations in incorporating enzymes in commercial diets

1. What is the target ingredient?

2. What enzyme activities are required?

3. What is the stability of the enzyme source during feed processing and within the digestive tract?

4. Will an enzyme cocktail be more effective than a single type of enzyme?

5. Do different CEPs have the same efficacy?

6. Should the enzymes be added to the dry mix or applied as a liquid spray?

7. What are the costs and benefits of using the enzymes?

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Effects of enzyme supplementation and irradiation of rice bran on the performance of Leghorn and broiler chicks

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A total of 40–45 × 10^6 t of rice bran is produced annually, mainly in the Far East and Southeast Asia (Farrell 1994). It is used largely as animal feed. Only a limited amount of rice bran is currently used for food or oil extraction (Takano 1993). Rice bran is a valuable feedstuff because it is rich in B vitamins, fat, and protein and compares favourably with other cereal grains in amino acid composition (Warren and Farrell 1991). It has, however, a high content of fibre (Warren and Farrell 1990a–d; Farrell 1994) that is rich in the hemicelluloses containing highly branched arabinoxylans (Erbingerova et al. 1994). A significant decline in chick performance with increasing rice-bran content in the diet has been noted in several studies (Warren and Farrell 1990a–d; Farrell 1994; Madrigal et al. 1995). Studies on the use of feed enzymes to improve the nutritive value of rice bran have been limited (Farrell 1994). Nevertheless, rice bran with a high content of arabinoxylans may respond to enzymes that can hydrolyze this complex carbohydrate. Irradiation has also been shown effective in improving the nutritive value of cereal grains (Campbell et al. 1983; Campbell et al. 1986; Campbell et al. 1987).

The purpose of this study was to determine the effects of irradiation and of the addition of an enzyme preparation high in xylanase activity on the nutritive value of rice bran fed to growing chicks. The data here have already been published (Wang at al. 1995, 1996).

Materials and methods

Enzymes

Crude-enzyme preparation (RM-1, from *Trichoderma longibrachiatum*) was supplied by Finnfeeds International Ltd, Marlborough, Wiltshire, United Kingdom. It contained 3 450 U xylanase activity / g and 900 U β-glucanase activity / g. It also

Table 1. Chickens and experimental designs used in the three experiments.

	Type of chicken	Period (d of age)	Diet	Design (dietary treatment)
Exp. 1	Leghorn	6–13	Wheat–soybean	3 (0, 1, and 10 g RM / kg feed) × 3 (0, 10, and 50 kGy/s); 6 replicates of 6 birds each
Exp. 2	Leghorn	4–11	Corn–soybean	3 (0, 1, and 10 g RM / kg feed) × 3 (0, 25, and 50% RB); 6 replicates of 6 birds each
Exp. 3	Broiler	5–12	Corn–soybean	3 (0, 1, and 10 g RM / kg feed) × 3 (0, 25, and 50% RB); 6 replicates of 5 birds each

Note: RM, type of enzyme (RM-1, from *Trichoderma longibrachiatum*); kGy, radiation intensity; RB, rice bran.

contained pectinase, carboxymethylcellulose cellulase, β-xylosidase, acetyl esterase, and other enzyme activities.

Rice brans

Rice brans A, B, and C were from Malaysia, and Chinese rice bran was provided by Nanjing Agricultural University, Nanjing, China. The proportions of protein (N × 6.25) and fat (ether extract) in rice bran B, in a mixture of A and C, and in Chinese rice bran were 11.3 and 14%, 11.8 and 15.9%, and 14.6 and 16.4%, respectively. Malaysian rice bran in experiment 1 was irradiated with 10 MeV, using a model I-10/1 electron accelerator (Atomic Energy of Canada Ltd, Pinawa, Manitoba), at average doses of 0, 10, and 50 kGy/s.

Chickens and experiment design

The chickens and experimental designs used in the three experiments are outlined in Table 1.

Experimental diets

The experimental diets were formulated to meet National Research Council requirements for Leghorn and broiler chickens (NRC 1994). The calculated protein and metabolic-energy levels in the diets for Leghorn chickens (experiments 1 and 2) were 180 g/kg and 12.2 MJ/kg, respectively. All diets were formulated to be isoenergetic and isonitrogenous. However, the diets for broiler chicks in experiment 3 were formulated to contain 220 g protein / kg and to be nearly but not entirely isoenergetic, as the calculated energy values of the diets containing 25 and 50% rice bran were 2.4 and 4.8% lower than that of the reference corn–soybean-meal diets (12.6 MJ/kg).

Chicken performance was expressed in terms of feed consumption, body-weight (BW) gain, and feed–gain ratio. The size of the digestive organs (weight and length) and the vent-pasting scores of broilers in experiment 3 were recorded at 15 d of age (Marquardt et al. 1994).

Analysis of variance (ANOVA) was conducted for all data using the general linear model procedure of the SAS Institute, Inc. (1986). Linear contrasts and Duncan's multiple-range test were used for comparison of treatments. Linear contrasts were also used to compare performance values for chicks fed diets containing Chinese rice bran and for those fed the wheat–soybean-meal control diet.

Results

Experiment 1

ANOVA of experiment 1 data indicated that the addition of enzymes to the rice-bran diets improved BW gain ($P < 0.05$) and the feed–gain ratio ($P < 0.01$) but did not affect feed consumption ($P > 0.05$). Irradiation of the rice bran did not affect chick performance ($P > 0.05$). The improvements in BW gain with the addition of enzymes to the diets containing the Malaysian rice bran were 5.6 ($P = 0.04$) and 9.6% ($P < 0.01$) with 1 and 10 g enzyme / kg feed, respectively, and the corresponding improvements in the feed–gain ratio were 3.2 ($P = 0.005$) and 5.4% ($P < 0.001$). Linear-contrast analysis, in contrast to ANOVA, indicated that feed consumption in chicks fed the diets with high enzyme levels also increased (3.7%, $P = 0.04$). Adding enzymes to diets containing Chinese rice bran did not affect chick performance ($P > 0.05$). The performance of chicks fed the Malaysian rice bran without enzymes and the Chinese rice bran with or without enzymes was generally the same ($P > 0.05$) as that of chicks fed the wheat–soybean-meal control diet. In contrast, the BW gains of chicks fed the Malaysian rice bran supplemented with 1 or 10 g enzyme / kg feed were 6.9 ($P = 0.05$) and 10.9% ($P = 0.004$) higher than those of chicks fed the wheat–soybean control diet. The corresponding improvements in the feed–gain ratio were 3.2 ($P = 0.055$) and 5.4% ($P = 0.002$). These results, which were obtained during a short experimental period, indicate that the performance of Leghorn chicks was, in some cases, improved by adding enzymes to diets with a high content of rice bran (40%) and that the performance of chicks fed diets with or without enzyme supplementation was similar or superior to that of chicks fed the wheat–soybean control diet. Irradiation of rice bran did not affect chick performance.

Experiment 2

Linear contrasts indicated that enzymes had a significant effect on the performance of Leghorn chicks when they were fed the rice bran diets ($P < 0.05$; see contrasts 7, 8, and 9, Table 2) but not when they were fed the corn control diet ($P > 0.05$; see contrasts 1 and 2). For example, the overall improvements were 7.1% ($P = 0.04$) for BW gain and 4.3% ($P = 0.05$) for feed–gain ratio when the results for diets 5, 6, 8, and 9 ($RB_{1,10}$, that is, rice bran with 1 or 10 g enzyme/kg) were contrasted with those for diets 2 and 3 (RB_0, that is, rice bran with no enzymes). Comparison of the corn diets (C_1–C_0 and C_{10}–C_0) indicated that dietary enzyme had no effect on chick performance ($P > 0.05$). Also, the performance of Leghorn chicks fed rice bran with no enzymes ($250RB_0$ and $500RB_0$) was the same ($P > 0.05$) as that of chicks fed the control diet (C_0) ($P > 0.05$; see contrasts 3 and 4) but was superior ($P < 0.05$) to that of chicks fed the control diets in several of the comparisons when enzymes were added to the two bran diets ($250RB_{1,10}$ and $500RB_{1,10}$; see contrasts 5 and 6). For example, the diets containing 25% rice bran plus enzymes ($250RB_{1,10}$) yielded values for feed consumption, BW gain, and feed–gain ratios that were 7.0 ($P = 0.01$), 17 ($P = 0.01$), and 8.7% ($P = 0.05$) higher than those obtained with the control diet (C_0). The differences were less pronounced when the diet contained 50% rice bran (see contrasts 4 and 6). These results again indicate that Leghorn chicks fed diets containing high levels of rice bran (25 or 50%) had performance values similar to, or better than, those fed a corn control diet and that adding enzymes to rice-bran diets appeared to improve chick performance.

Experiment 3

ANOVA of experiment 3 data indicated that there were main effects ($P < 0.05$) for both diet and enzyme treatments (Table 3). In general, feed consumption and BW gain decreased and the feed–gain ratio increased when 25 and 50% rice bran were included in the diets, and the effects were much greater than the corresponding changes in the energy value of the diets. High concentrations of enzymes in the diets improved the feed–gain ratio by 4%. For chicks fed diets 1, 3, 7, and 9, the performance data determined on day 15 followed the same trend as those determined on day 12 (data not shown). The inclusion of 50% rice bran in the diets markedly increased ($P < 0.05$) the relative size and length of all segments of the digestive tract. The average increase in the weight of the digestive tract was 40% (from 9.2 g/100 g BW to 12.9 g/100 g BW), and the corresponding increase in its length was 38% (from 39.8 cm/100 g BW to 55.1 cm/100 g BW).

Table 2. Selected linear contrasts of performance levels for Leghorn chicks fed nine different diets containing three concentrations of enzymes (experiment 2).

Contrast No.	Contrasts		Percentage increase or decrease in first value in parentheses relative to second value in parentheses					
			Feed consumption (g) [b,c]		BW gain (g) [b,c]		Feed–gain ratio [b,c]	
	Description	Diet No. [a]	%	*P*	%	*P*	%	*P*
	Control diet (C) with enzymes (C_1 or C_{10}) versus control diet without enzymes (C_0) [d]							
1	C_1–C_0	4–1	1.0 (80.2 vs 79.4)	NS	2.0 (33.8 vs 33.0)	NS	−1.7 (2.38 vs 2.42)	NS
2	C_{10}–C_0	7–1	−1.0 (78.5 vs 79.4)	NS	2.5 (33.8 vs 33.0)	NS	−3.7 (2.33 vs 2.42)	NS
	Rice bran (RB) at 250 or 500 g / kg diet with enzymes (RB_1 or RB_{10}) or without enzymes (RB_0) versus control diet without enzyme (C_0) [d]							
3	$250RB_0$–C_0	2–1	3.7 (82.3 vs 79.4)	NS	9.1 (36.0 vs 33.5)	NS	−4.5 (2.31 vs 2.42)	NS
4	$500RB_0$–C_0	3–1	1.0 (80.2 vs 79.4)	NS	3.3 (34.1 vs 33.5)	NS	−2.5 (2.36 vs 2.42)	NS
5	$250RB_{1,10}$–C_0	5,8–1	7.0 (85.0 vs 79.4)	0.01	17.0 (38.6 vs 33.5)	0.01	−8.7 (2.21 vs 2.42)	0.05
6	$500RB_{1,10}$–C_0	6,9–1	4.0 (82.6 vs 79.4)	NS	10.3 (36.4 vs 33.5)	0.05	−5.8 (2.28 vs 2.42)	NS
	Rice bran with enzyme (RB_1 or RB_{10}) versus rice bran with no enzyme (RB_0) [d]							
7	RB_1–RB_0	5,6–2,3	9.4 (82.8 vs 81.2)	0.41	7.0 (37.5 vs 35.0)	0.07	−5.6 (2.21 vs 2.33)	0.026
8	RB_{10}–RB_0	8,9–2,3	4.4 (84.8 vs 81.2)	0.06	7.2 (37.6 vs 35.0)	0.07	−3.0 (2.27 vs 2.33)	0.065
9	$RB_{1,10}$–RB_0	5,6,8,9–2,3	3.2 (83.8 vs 81.2)	0.12	7.1 (37.5 vs 35.0)	0.04	−4.3 (2.24 vs 2.33)	0.05

Note: BW, body weight; NS, not significant.

[a] Diets 1–9 contained different amounts of Malaysian rice bran (0, 25, or 50% per kg) and different amounts of enzymes (0, 1, or 10 g/kg). Dietary treatments (g RB / kg + g enzyme / kg): (1) 0 + 0; (2) 250 + 0; (3) 500 + 0; (4) 0 + 1; (5) 250 + 1; (6) 500 + 1; (7) 0 + 10; (8) 250 + 10; (9) 500 + 10.

[b] Values in parentheses represent feed consumption (g/chick over a 7-d period), BW gain (g/chick over a 7-d period), and the feed–gain ratio for diets listed under Contrasts.

[c] In general, there were no significant differences for feed consumption, BW gain, or feed–gain ratio for the following contrasts: $250RB_1$–$250RB_0$ (5–2), $250RB_{10}$–$250RB_0$ (8–2), $500RB_1$–$500RB_0$ (6–3), and $500RB_{10}$–$500RB_0$ (9–3). However, among these comparisons there was a significant difference in BW gain for $250RB_{10}$–$250RB_0$ (8.3%, 39.0 vs 36.0, $P < 0.05$) and in the feed–gain ratio for $500RB_1$–$500RB_0$ (−6.8%, 2.20 vs 2.36, $P < 0.04$).

[d] The subscripts 0, 1, and 10 indicate no enzyme, 1 g enzyme / kg diet, and 10 g enzyme / kg diet, respectively.

The inclusion of 10 g enzyme / kg feed had no effect ($P > 0.05$) on the weight of the digestive organs of chicks fed the corn control diet, but enzymes in the diet containing 50% rice bran decreased ($P < 0.05$) the weight of the proventriculus (11%), gizzard (13%), colon (8%), and total gastrointestinal tract (7%). The relative length of the digestive tract was unaffected ($P < 0.05$) by enzyme treatment. The degree of vent pasting in 15-d-old chicks also increased ($P < 0.05$) when rice bran was added to the diet. These chicks had been fed the diet for 10 d. No apparent vent pasting was observed in experiment 2 when Leghorn chicks were fed the diet containing 50% rice bran for 7 d (5–12 d of age). These data also indicate that adding enzymes to the diet improved broiler chick performance and reduced the size of the gastrointestinal tract and the incidence of vent pasting.

Table 3. Performance of broiler chicks between 5 and 12 d of age when fed diets containing three concentrations of enzymes and three concentrations of rice bran (experiment 3).

	Feed consumption (g/bird over 7 d)	BW gain (g/bird over 7 d)	Feed–gain ratio [a]	Frequency of vent pasting (%)
Treatment		Main effect [b]		
Rice bran (RB) (g / kg diet)				
0	212*a*	150*a*	1.42*a*	0*a*
250	212 (0)*ab*	143 (5)*b*	1.49 (−5)*b*	14*b*
500	195 (8)*b*	124 (17)*c*	1.57 (−11)*c*	44*c*
Enzyme (E) (g / kg diet)				
0	206	137	1.51*b*	30*b*
1	208	137	1.52 (0)*b*	14 (−53)*a*
10	206	142	1.45 (−4)*a*	14 (−53)*a*
Pooled SEM	2.41	1.89	0.016	3.3
ANOVA		Probability		
E	0.84	0.15	0.01	0.002
RB	0.0001	0.0001	0.0001	0.0001
E × RB	0.61	0.51	0.14	0.03

Note: ANOVA, analysis of variance; BW, body weight.

[a] The vent-pasting values for each RB and E treatment were 0% for 0RB, 0E; 23% for 250RB, 0E; 67% for 500RB, 0E; 0% for 0RB, 1E; 10% for 250RB, 1E; 33% for 500RB, 1E; 0% for 0RB, 10E; 10% for 250RB, 10E; and 33% for 500RB, 10E. Values were recorded on day 15 of the experiment.

[b] Values in parentheses are the percentage increases or decreases obtained relative to the diet with no rice bran.

a–c, Means for each main effect within a column followed by a different letter are significantly different ($P < 0.05$).

Discussion

The results of this study demonstrate that diets containing high quantities of rice bran (25–50%) without enzymes yielded BW gains and feed–gain ratios in Leghorn chickens similar to those obtained with corn- or wheat-based control diets. In contrast, similar amounts of rice bran in diets fed to broiler chicks decreased the feed consumption and BW gains and increased the feed–gain ratio relative to those obtained with broilers fed the corn-based diet. This effect was more pronounced for the diet with a high concentration of rice bran (25% versus 50%). Data from the literature indicate that usually the weight of broiler chicks decreases as the content of full-fat rice bran in the diet increases, with the magnitude of the effect varying considerably among experiments and depending in part on the effect that the diet has on feed consumption (Kratzer et al. 1974; Warren and Farrell 1990b; Farrell 1994). Similarly, the feed-conversion rates (FCRs) are usually poorer with increasing concentration of rice bran in the diet; the point at which this occurs, however, varies. Warren and Farrell (1990b) observed no change in FCRs up to 400 g rice bran / kg diet in some experiments, but in other experiments a poor FCR was observed at 200 g/kg: clearly, some factor(s) in rice bran affects chick performance in an unknown manner. Variations in the fibre, protein, and oil contents, rancidity of the lipid fraction, and other constituents in rice bran may be responsible for this effect.

In addition to affecting the performance of broiler chickens, rice bran substantially increased the size of the gastrointestinal tract. Similar increases were reported in chickens fed a diet high in soluble (Scout barley) or insoluble fibre (Bedford barley) (Brenes et al. 1993). Studies by Jones et al. (1985), Sircar et al. (1983), and Rompala and Madsen (1989) also showed that diets high in roughage increased the size of the gastrointestinal tract in other species of animals. Part of this effect in broiler chickens, however, may have been attributable to differences in mature size of the birds in the different treatment groups: younger, less-developed birds tend to have relatively larger intestines than more mature birds (Sreemannarayana et al. 1989). These observations nevertheless suggest that the high content of neutral detergent fibre in rice bran (200 g / kg dry matter) (Warren and Farrell 1990d) may have been responsible in the current study for the increased size of the gastrointestinal tract. The arabinoxylans are probably mainly responsible for this effect, as they constitute the bulk of the hemicelluloses in rice bran (Erbingerova et al. 1994).

Previous studies showed that irradiation improves the nutritive value of cereals such as oat groats (Campbell et al. 1986; Campbell et al. 1987) and rye (Campbell et al. 1983). The beneficial effects of irradiation were attributed to the partial breakage of the polymeric structure of the viscous carbohydrates in the two

cereals. This breakage decreased their viscosity and produced a corresponding reduction in their antinutritive effects. In contrast, irradiation of rice bran at 10 or 50 kGy/s in the current study did not improve its nutritive value. This lack of response was probably not caused by a failure to break the rice-bran polysaccharides, as the dose was sufficient to bring this about. Irradiation may have been ineffective because of the absence of viscous carbohydrates in rice bran. Under such conditions, no change in viscosity will occur with irradiation. Therefore, the corresponding improvements associated with these changes would not be seen.

Adding a crude-enzyme preparation to diets containing high concentrations of rice bran produced equivocal results. For example, the high concentration of enzymes (10 g/kg feed) produced no beneficial response in Leghorn chicks fed the Chinese rice-bran diet, but it improved BW gain and the feed–gain ratio by 7 and 3.4%, respectively, in Leghorn chicks fed the Malaysian rice-bran diets. The high concentration of enzymes added to broiler chick diets containing the Malaysian rice bran not only improved the feed–gain ratio but also reduced the incidence of vent pasting and decreased the size of the gastrointestinal tract. The results collectively indicate that enzyme supplementation of diets containing rice bran can improve chick performance in some cases. The reason for the completely different responses to enzyme-treated rice brans obtained from two different regions in Asia was not established. This difference may in part be caused by differences in processing methods or by varietal or environmental differences during growth. The limited proximate analysis carried out on the rice brans would indicate that the difference was not caused by the contents of crude protein or fat. This study also demonstrated that the BW gain and feed–gain ratio for the Leghorn chicks fed the enzyme-supplemented rice-bran diets, especially the diet containing 25% rice bran, were superior to those of the chicks fed the corn control diet. Only a few studies have been reported on the use of enzymes in poultry diets containing rice bran (Farrell 1994). Experiments with lipase and two modified enzyme mixtures that targeted the cell-wall content showed essentially no improvement in the nutritive value of rice bran in broiler starter diets. The improvement noted in the current study was probably due to the effects of the xylanases or possibly of the ß-glucanases on the hemicellulose fraction of the rice bran.

All of the above studies were of relatively short duration (7 d), and it is not known whether a similar pattern of response would be obtained over a much longer period. Nevertheless, this study has shown that high concentrations of rice bran fed to Leghorn chicks for a short time, especially in association with an enzyme preparation high in xylanase and ß-glucanase activities, produced BW gains and feed–gain ratios that were equal to, or superior to, those produced by

corn- or wheat-based diets. However, broiler chicks were not able to utilize rice-bran diets as well as they utilized the control diets. The high fibre content of the rice bran may have been partially responsible for this effect. Further research is needed to identify factors that affect the efficacy of enzyme preparations added to diets containing rice bran, to establish the degree of corresponding response of these treatments in different classes of chicks and over longer periods, and to determine the influence of the type of rice bran and type of enzyme preparation on the response. Such studies would help to clarify the results of this study and to determine the optimum levels of rice bran that can be used in different diets in the presence and absence of the appropriate enzyme mixture.

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Performance improvements following enzyme supplementation of wheat- and barley-based poultry diets

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At the beginning of 1995/96, the US stock of maize (corn) fell to a record low of 10.6×10^6 t, only 5% of the volume used. This situation was due primarily to reduced production of corn in the United States in 1995 (Meat International 1996). Livestock-feed production has been rapidly increasing in Asia. Only 2 years ago, China was an exporter of maize, but now it must import this product, primarily from the United States, to sustain its rapidly growing livestock industry. Because of the worldwide shortage of maize, Chinese nutritionists may soon be forced to consider alternative raw materials to sustain continued growth in livestock production. The availability of wheat on world markets is forecast to increase (Elliott 1996). Wheat and barley are extensively used in poultry feeds in Australia, Europe, and Canada, but the poultry industry in Asia is relatively unaccustomed to using high proportions of these ingredients. This review deals with the use of wheat and barley in poultry diets, focusing on the improvements in the nutritive value of these ingredients that can be obtained with enzyme supplementation.

Broiler-feed formulation in China

A comparison of the nutrients and antinutritive factors in corn, wheat, and barley (Table 1) helps in understanding how enzymes improve the nutritional value of wheat and barley. The low energy values of wheat and barley are of concern to the formulators of diets in Asia, as energy costs are high. Feed formulation is therefore complex, and many factors need to be considered. For example, corn has less protein, lysine, tryptophan, and arginine than wheat or barley, but wheat may be deficient in available energy, biotin, and xanthophyll. On the other hand, wheat has improved pelleting characteristics, which means that there is no need for the pellet binders commonly used in corn-based feeds. Also worth considering are the

Table 1. Nutrients and antinutritive factors in corn, wheat, and barley.

	Corn	Wheat	Barley
Energy	3 350.00	3 120.00	2 620.00–2 640.00
Crude protein	8.50	11.50–14.00	9.50–11.00
Lysine	0.25	0.31	0.38
Methionine	0.18	0.17	0.17
Methionine + cysteine	0.37	0.42	0.39
Threonine	0.30	0.32	0.35
Tryptophan	0.06	0.40	0.12
Arginine	0.39	0.51	0.49
Crude fibre	2.20	3.00	5.50–6.40
Soluble β-glucan	—	0.70	2.70
Total β-glucan	—	0.70	4.40
Soluble pentosans	0.70	1.20	0.20
Total pentosans	4.20	6.60	5.70

different priorities of compounders within the Chinese poultry-feed industry. Commercial feed producers will be more sensitive to actual feed costs in a competitive market environment, whereas vertically integrated operations are more concerned with the cost per kilogram of broiler or egg. In the latter situation, a higher feed cost may be accepted if production is improved.

Antinutritive factors in wheat and barley

Wheat, barley, triticale, rye, and oats contain relatively high proportions of antinutritive carbohydrates known as nonstarch polysaccharides (NSPs). The intestinal viscosity caused by water-soluble NSPs dramatically reduces bird performance (Choct and Annison 1992b). The content of NSP in the diet is inversely related to the apparent metabolizable energy (AME) of wheat (Annison 1991) and positively correlated with gut viscosity (Bedford et al. 1991; Bedford and Classen 1992). Gut viscosity is inversely related to nutrient utilization and bird performance (Bedford et al. 1991; Annison 1992; Bedford and Classen 1992). Choct and Annison (1992a) demonstrated that the concentration of soluble arabinoxylan in broiler diets is positively correlated with the relative depression in AME, nitrogen retention, feed-conversion efficiency, and weight gain. Wheat diets containing 4% arabinoxylans decreased digestibility of starch, protein, and lipids by 14.6, 18.7, and 25.8%, respectively. Differences in content and composition of NSPs among barley or wheat varieties are associated with differential effects of these cereals

on poultry productivity. Barley varieties can be classified as having a "high" or "low" content of β-glucan, which is responsible for significant differences in biological responses when barley-based diets are fed to poultry (Campbell et al. 1989). Similarly, researchers investigating wheat in Australia have identified "low-AME" wheats (energy value of <12 975 kJ/kg) (Mollah et al. 1983; Rogel et al. 1987; Choct and Annison 1990). Adding enzymes to wheat- and barley-based poultry feeds to hydrolyze NSPs and reduce the negative effects of antinutritive factors, minimize variability, and therefore improve ingredient value is now a commonplace practice.

Although consideration of the benefits of exogenous enzymes has focused mainly on cereals (wheat, barley, and corn), other dietary components should also be considered. For example, diets containing barley as the major cereal source will also typically contain wheat or rice by-products and plant-protein meals. To target only the barley component of the diet with a β-glucanase is inappropriate: enzyme supplements should ideally contain adequate activities of xylanases, cellulases, and pectinases for secondary feed ingredients. In commercial situations, therefore, it is worth considering the practical value of using multienzyme complexes (targeting one feed) instead of substrate-specific enzymes (targeting one ingredient).

Responses to enzymes

Boilers

As a result of endo-xylanase and β-glucanase supplementation, the long backbones of the arabinoxylans and β-glucans are cleaved into shorter fragments, thereby reducing their viscosity (Gruppen et al. 1993). Supplementing broiler diets with combinations of xylanase and β-glucanase minimizes the adverse effects of NSPs and improves the nutritive value of the diet (Campbell et al. 1989; Francesch et al. 1989; Helander and Inborr 1989; Wiedmer and Völker 1989; Jansson et al. 1990; Bedford et al. 1991; Benabdeljelil 1992; Brufau et al. 1993; Jeroch and Dänicke 1993; Schurz et al. 1993; Vukić Vranješ and Wenk 1993; Benabdeljelil and Arbaoui 1994; Broz and Perrin-Voltz 1994; Broz et al. 1994; Marquardt et al. 1994; Veldman and Vahl 1994; Allen et al. 1995; Almirall et al. 1995; Choct et al. 1995; Classen et al. 1995; Fuente et al. 1995; Juin et al. 1995; Klünter, Devaud et al. 1995; Klünter, Weber et al. 1995; Langhout and Schutte 1995; Mohammed 1995; Partridge and Wyatt 1995; Schutte et al. 1995; Van der Klis et al. 1995; Vukić Vranješ and Wenk 1995; Dunn 1996).

One of the main reasons for supplementing wheat- and barley-based poultry diets with enzymes is to increase the available energy content of the diet. Increased availability of carbohydrates for energy utilization is associated with increased energy digestibility (Partridge and Wyatt 1995; Van der Klis et al. 1995).

The AME of wheat has been extensively studied and found to have a considerable range (9 500–16 640 kJ/kg) (Mollah et al. 1983; Rogel et al. 1987; Annison 1995; Choct et al. 1995; Ward 1995). Enzyme supplementation improves this range by enhancing carbohydrate digestibility, reducing gut viscosity, and improving fat utilization (Almirall et al. 1995). The improvements in AME resulting from enzyme supplementation are variable because of the variability in the NSP content of wheat. Classen et al. (1995), Schutte et al. (1995), and Van der Klis et al. (1995) reported improvements of 5–16, 3.1–4.5, and 4.5–12.4%, respectively, and in recent Australian trials, improvements of 3–30% were observed (unpublished data). The increase in AME with the use of enzymes is difficult to predict, as nutrient ratios, such as energy–protein, and other factors also play an important part in poultry-feed formulations. The AME value of wheat has been correlated with its content of water-soluble NSPs (Annison 1991), which in turn affects gut viscosity (Bedford et al. 1991). Unfortunately, NSP analyses are relatively lengthy processes, and in a commercial situation rapid testing of incoming grains is required. No chemical test or detectable physical characteristic can be used to rapidly predict the AME value of wheat or to estimate the improvements to be expected from the use of enzymes. This is part of the difficulty in trying to accurately estimate the energy content of wheat or barley in poultry feeds and compensate for the deficiency by adding enzymes.

The importance of energy compensation in feed formulation was demonstrated in a cost–benefit study in Australia in 1991 (unpublished data). A wheat-based diet was formulated with or without the 5% increase in AME value obtained with enzyme supplementation. Broiler growth, feed conversion, and AME improved because of the supplementation. Not compensating for the improvement in AME increased the calculated cost of supplemented feed. Nevertheless, as a result of improved growth and feed efficiency, the cost per kilogram broiler in the enzyme-treated group was 1.3% lower than that in the wheat-control group. Compensating for the additional energy further improved production characteristics and also reduced feed costs, giving a reduction in cost per kilogram broiler of 8.8% compared with the wheat control. Partridge and Wyatt (1995) cited similar benefits when allowances were made for the improvements in energy and amino acid digestibility. The problem facing the feed formulator is estimating the correct energy allowance for wheat-based diets. Typically, a conservative 5–6% upgrading of the AME of wheat is recommended for commercial situations. This allowance effectively improves the energy value of wheat to about 13 800 kJ/kg in a least-cost matrix, bringing the value of a wheat–enzyme combination closer to that for maize and allowing the use of less supplementary energy. Amino acid adjustments may also be made, as enzyme supplementation also improves protein digestibility

(Bedford et al. 1991; Partridge and Wyatt 1995). Typically, the digestibility of amino acids should be expected to increase by 10% with added enzymes (Bedford et al. 1991; Ward 1995).

A great deal of literature deals with broiler-growth and feed-conversion responses in wheat- and barley-based diets. The addition of barley to broiler feed has been considered impractical because of barley's limited energy value and high β-glucan content, which impairs growth and feed efficiency and leads to a high incidence of wet or sticky droppings. Storing barley reduces but fails to eliminate the antinutritive effects of the β-glucan (Brufau et al. 1993). Supplementary β-glucanase has been shown to minimize the negative effects of barley β-glucan (Wiedmer and Völker 1989; Jansson et al. 1990; Brufau et al. 1992; Brenes et al. 1993; Broz et al. 1994; Partridge and Wyatt 1995). Trials have shown that barley–enzyme combinations can result in broiler performances comparable to those attained with corn (Marquardt et al. 1994; Almirall et al. 1995; Fuente et al. 1995; Partridge and Wyatt 1995). The difficulties in predicting responses because of the variable content of β-glucan in barley were highlighted by Almirall et al. (1995). When corn-based and barley-based broiler diets were compared, the feed-conversion efficiency of broilers fed barley plus enzymes was the same as that for broilers fed the corn diet. Daily gain was affected by the variety of barley: "low-viscosity" barley yielded values not significantly different from those obtained for chicks fed corn, but the gain with "high-viscosity" barley was 8.7% less.

Wheat is the most common cereal used in poultry feed in Australia, Canada, and the United Kingdom. A considerable amount of research has been done on broilers' responses to wheat-based diets. Commercial broiler feeds typically contain in excess of 60% wheat, and the inclusion of xylanase-based enzymes in these diets is now commonplace. Positive effects on AME, weight gain, feed conversion, protein digestibility, fat digestibility, and litter condition were observed when broiler diets containing a high proportion of wheat were supplemented with enzymes (Helander and Inborr 1989; Jansson et al. 1990; Graham and Harker 1991; McNab et al. 1993; Schurz et al. 1993; Veldman and Vahl 1994; Classen et al. 1995; Juin et al. 1995; Klünter, Weber et al. 1995; Langhout and Schutte 1995; Rajmane et al. 1995). The growth and feed-conversion efficiency obtained with wheat–enzyme combinations can exceed those obtained with corn-based diets (Juin et al. 1995; Marquardt et al. 1994; Partridge and Wyatt 1995). Partridge and Wyatt (1995) undertook a step-wise replacement of corn with wheat in broiler diets. Intestinal viscosity increased with only 20% dietary wheat, but production parameters were not significantly affected until the content of wheat exceeded 40%. At this and at the 60% wheat-replacement level, the feed-conversion efficiency and weight gain obtained with added enzymes exceeded

those obtained with the corn control. Performance is directly related to the composition of the diet, and with the correct formulations, wheat and barley can be more valuable feed ingredients than corn.

Responses to enzyme supplementation depend on the bird's age, which is apparently related to both the type of gut microflora present and the physiology of the bird. Older birds, because of the enhanced fermentation capacity of the microflora in their intestines, have a greater capacity to deal with negative viscosity effects (Allen et al. 1995; Choct et al. 1995; Vukić Vranješ and Wenk 1995).

The dry-matter content of the litter of wheat- or barley-fed broilers is improved (reduced sticky droppings) by adding enzymes to their diets (Wiedmer and Völker 1989; Jansson et al. 1990; Mohammed 1995). The improved litter condition reduces ammonia buildup in sheds and reduces the incidence of hock burns and breast blisters. Also, birds fed high-barley or high-wheat diets have been shown to have elevated intestinal weight, which negatively affects the carcass yield. This negative effect is reduced after supplementation with the appropriate enzymes (Francesh et al. 1989; Jeroch and Dänicke 1993).

Replacing maize with wheat reduces the total xanthophyll content of the feed, thus reducing the pigmentation of the broiler. Combinations of supplementary xanthophylls are required in maize-based broiler diets to satisfy China's consumer demand for chickens with coloured skin (Bird 1994a and b). Therefore, these supplements must be used if maize is excluded from chickens' diets.

Laying hens

The responses of laying hens to enzyme-supplemented feeds are also well documented. Typically, enzymes added to layer feed appear to have little effect on egg mass but improve feed efficiency (Benabdeljelil and Arbaoui 1994; Vukić Vranješ and Wenk 1995), energy utilization (Wyatt and Goodman 1993; Vukić Vranješ and Wenk 1995), and laying rate (Poultry International 1996). Wyatt and Goodman (1993) reported that corn-fed layers exhibited better feed efficiency than those fed enzyme-supplemented barley-based diets. Nevertheless, enzyme supplementation improved the utilization of barley diets. Increased energy utilization in laying hens appears to be due to microbial fermentation of solubilized NSPs (Vukić Vranješ and Wenk 1995) and the subsequently higher absorption of volatile fatty acids (Choct et al. 1995). Wet litter arising from the use of barley and newly harvested wheat can result in an increased incidence of dirty egg shells and in ammonia buildup in poultry barns. Adding enzymes to both wheat- and barley-based diets has been shown to reduce the moisture content of fecal matter in layers (Marquardt et al. 1994). This means that barley can effectively be used if diets are supplemented with the appropriate enzymes.

Egg-yolk pigmentation should also be considered if maize is excluded from the diet. Wheat and barley contain very low levels of xanthophylls; if these grains are fed to layers, the yolk will be practically colourless and consumers will reject them. The diets therefore need to be supplemented with dietary carotenoids. The quantities required are well documented because of the extensive use of wheat and barley in layer feeds (Bird 1994a and b). Pigments are fat-soluble compounds and are therefore less efficiently absorbed in the presence of highly viscous compounds such as those found in barley-based diets (Benabdeljelil and Arbaoui 1994; Poultry International 1996).

Ducks

Nutritionists often avoid using a high proportion of corn in duck feed because of the possibility of aflatoxin contamination. Wheat, barley, or rice products are feasible feed sources for meat and laying ducks. In two studies, in France and Germany (Roche, unpublished data), improvements in meat-duck daily gain (5.8 and 3.3%) and feed conversion (7 and 1.8%) and a reduction in litter moisture were obtained when enzymes were added to the diet. Improvements in live-weight gain reduced the growth period by 5 d for 3.65- and 3.5-kg ducks, which would allow significant savings in commercial duck production.

Synergy with antibiotics

Researchers have observed a synergistic response to antibiotic and enzyme supplements in broiler feeds containing wheat (Schurz et al. 1993; Broz et al. 1994; Allen et al. 1995; Choct et al. 1995; Langhout and Schutte 1995; Pijsel 1996) and in those containing barley (Broz et al. 1994; Vukić Vranješ and Wenk 1995). Typically, weight-gain and feed-conversion responses are observed for each supplement, with a degree of nonadditive synergism. The importance of established gut microflora in the digestion of fibre is greater in older birds than in younger birds, with the positive effects of enzymes in layers appearing to require active microflora to degrade the NSP solubilized by enzyme action (Choct et al. 1995). The beneficial effects of enzymes in barley-fed layers can be eliminated by the addition of flavomycin, a compound that reduces the fermentative capability of gut microflora (Vukić Vranješ and Wenk 1995). Allen et al. (1995) reported that the inclusion of antibodies in the gut of broilers not only improved production parameters, including weight gain, but also increased the viscosity of digesta. This result is the opposite of that observed when enzymes are added to the diet. These data indicate that both high and low viscosities are associated with improved nutrient utilization. Presumably, enzymes and antibiotics have different modes of action and therefore increase nutrient utilization in a different manner.

Conclusion

The current and future shortfall in supplies of corn will force nutritionists to consider alternative feed ingredients. The use of wheat and barley in poultry diets is not new, and the value of these ingredients has been improved by the use of enzyme supplements. Unfamiliarity with wheat and barley has limited their use as raw materials in poultry diets in Asia. Many of the nutritionists are trained in the use of traditional corn and soybean diets, and they often feel uncomfortable using an alternative cereal to make up 50–70% of a diet. The availability of commercial carbohydrate-degrading enzymes and the use of appropriate formulation techniques have made it feasible to use wheat and barley in poultry feeds. Adding carbohydrases to poultry diets has improved the nutritive value of some cereals and legumes, allowing for a reduction of the supplementary energy in the diet and the inclusion of higher proportions of less digestible cereals (rye) or more problematic ones (new barley or wheat). Advantages to poultry producers include improved weight gain, feed-conversion efficiency, and litter condition. Adding enzymes to these cereals has been shown to improve performance to levels at least as high as those obtained with corn-based diets. The unrestricted use of wheat or barley in poultry diets is therefore possible, with the amounts used depending on the supply and cost of the raw materials.

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A simple model equation for predicting chick response to enzymes

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The benefits of adding enzymes to the diets of nonruminant animals, particularly poultry, are well established (Campbell and Bedford 1992). Enzymes have been shown to improve performance and nutrient digestibility when added to poultry diets containing cereals, such as barley (Hesselman et al. 1982; Hesselman and Åman 1986; Friesen et al. 1992; Marquardt et al. 1994), oats (Friesen et al. 1992), rye (Fengler and Marquardt 1988; Fengler et al. 1988; Friesen et al. 1991, 1992; Bedford and Classen 1992; Marquardt et al. 1994), and wheat (Fengler et al. 1988; Friesen et al. 1991; Marquardt et al. 1994), and to those containing pulses, such as lupins (Brenes et al. 1993). The beneficial effects of adding enzymes have been attributed to a reduction in viscosity of digesta in the intestine (Antoniou et al. 1981; Hesselman and Åman 1986; Fengler et al. 1988; Bedford and Classen 1992; Marquardt et al. 1994).

Although enzymes have been widely used in animal feeds, no suitable simple model has been developed to predict their effects on animal performance. The objective of this study was to determine whether the response of chicks to dietary enzymes can be predicted using a simple model equation. In this study, rye grain was selected as one of the cereals because it contains high levels of viscous arabinoxylans (Antoniou et al. 1981), which are efficiently hydrolyzed by xylanases (Fengler and Marquardt 1988; Fengler et al. 1988; Marquardt et al. 1994). Wheat was used to replace rye, as it has much lower levels of these viscous compounds. In addition, data from the literature were analyzed. The research from this study has been published (Zhang et al. 1996).

Materials and methods

Materials and methods were outlined in detail in a previous publication (Zhang et al. 1996).

Table 1. Performance of Leghorn chicks fed a rye-based diet containing different amounts of enzymes (experiment 1).

Enzyme activity in diet (U/kg)	Weight gain (g / 6 birds)			Feed–gain ratio		
	Week 1	Week 2	Weeks 1 + 2	Week 1	Week 2	Weeks 1 + 2
0	196*d*	345*d*	541*e*	2.64*a*	2.30*a*	2.42*a*
88	242*c*	360*d*	602*d*	2.32*b*	2.27*ab*	2.29*b*
262	265*b*	363*cd*	628*cd*	2.20*c*	2.26*ab*	2.24*c*
788	276*b*	372*bc*	648*bc*	2.10*cd*	2.23*b*	2.18*d*
2 363	276*b*	390*ab*	666*ab*	2.15*cd*	2.18*c*	2.16*d*
7 090	296*a*	399*a*	695*a*	2.07*d*	2.18*c*	2.13*d*
Pooled SEM	5	8	11	0.03	0.02	0.02
Type of response due to enzyme activity	Probability					
Linear	0.000 1	0.000 1	0.000 1	0.000 1	0.000 1	0.000 1
Quadratic	0.000 1	0.017	0.000 1	0.000 1	0.000 2	0.000 1
Cubic	0.000 1	0.43	0.000 2	0.000 1	0.40	0.000 1

Note: Leghorn chicks were fed the experimental rye-based diet from 7 to 21 d of age. The diet contained 60% rye and 40% other ingredients, including various amounts of an enzyme preparation high in xylanase activity. SEM, standard error of the mean.

a–e, Means within a column not followed by the same letter are significantly different ($P < 0.05$).

Results and discussion

General observations (experiment 1)

Table 1 shows that the amount of enzyme preparation in the diet had a marked effect on the performance of Leghorn chicks for all periods ($P \leq 0.000\,2$). The overall improvements in weight gain and feed–gain ratio during the 2-week period were 28 and 12%, respectively ($P < 0.05$). Weight-gain response and feed–gain-ratio response were greater during week 1 (51 and 22%, respectively, $P < 0.05$) than during week 2 (16 and 5%, respectively, $P < 0.05$). The response to enzyme treatment had linear ($P \leq 0.000\,1$), quadratic ($P \leq 0.017$ to $0.000\,1$), and in some cases cubic ($P \leq 0.000\,2$, except for data from week 2) components. This suggests that the response was not directly proportional to the amount of enzyme added. For example, in week 1, enzyme added at the rate of 88 U/kg diet increased weight gain by 23% ($P < 0.05$), whereas increasing the amount of enzyme from 2 363 U/kg diet to 7 090 U/kg diet (an increase of 4 727 U) improved weight gain by only an additional 7% ($P < 0.05$). Similar trends were observed in all other comparisons. Overall, the response to enzyme treatment was not proportional

to the amount of enzyme added to the diet, and the response was much greater in week 1 than in week 2.

Prediction model from curve-fitting analyses (experiment 1)

The data from experiment 1 were subjected to different types of regression analysis (Table 2). When the data were subjected to linear-, quadratic-, and cubic-regression analyses, the amount of enzyme added to the diet was generally not significantly correlated ($P > 0.05$) with chick performance for week 1, but it was for week 2 ($P \leq 0.06$), during which the responses to enzyme treatment were the lowest (Table 1). However, when the enzyme-concentration data were converted into their logarithmic values and subjected to linear-regression analysis, all the log-linear values were significant ($P \leq 0.005$), with all regression values (r^2) being greater than 0.88. The r^2 values for weight gain and feed–gain ratio for the combined data from weeks 1 and 2 were all 0.99 ($P < 0.000\,1$). Although some numerical improvements were obtained using quadratic and cubic terms, these were generally not significant improvements ($P > 0.05$).

The log-linear model is a prediction equation (Table 3) that is simple to interpret, with the performance of chicks being a linear function of the logarithm of the amount of enzyme added to the diet. The model predicts that a 10-fold increase in enzyme concentration in the diet will result in a 2-fold, not a 10-fold, improvement in animal performance (that is, an increase in enzyme from 10 of these relative-concentration units to 100 of them represents corresponding logarithmic values of 1 and 2). It is obvious from this relationship that relatively small amounts of enzyme can have a dramatic effect on performance, whereas much larger amounts are required for each additional incremental improvement.

In regression analyses, an appropriate intercept value must be selected for diets that do not contain any added enzyme (0 enzyme) because the value for the logarithm of 0 cannot be calculated. Because no value can be calculated from log 0, any value close to the 0 value can be substituted for the 0 value (Cody and Smith 1991). However, the substitution of various small values for the 0 amount of enzyme can result in quite different r^2 values. Under such conditions, a value for 0 should be selected that is close to 0 and yields the largest r^2 and lowest error mean square. The value used to represent the diet with 0 enzyme supplement was 3.2 U/kg diet (see footnotes to Table 3).

The validity of this approach can be proven mathematically, as outlined below, using the following general equation:

$$Y = A + B \log X \qquad [1]$$

Table 2. Relationship between chick performance and units of enzyme activity added to the diet (experiment 1).

Type of regression analysis	Weight gain						Feed–gain ratio					
	Week 1		Week 2		Weeks 1 + 2		Week 1		Week 2		Weeks 1 + 2	
	r^2	P	r^2	P	r^2	P	r^2	P	r^2	P	r^2	P
Linear	0.44	0.15	0.73	0.03	0.56	0.09	0.30	0.26	0.62	0.06	0.44	0.15
Quadratic	0.59	0.26	0.94	0.02	0.74	0.13	0.48	0.38	0.96	0.008	0.68	0.18
Cubic	0.83	0.24	0.96	0.06	0.88	0.17	0.81	0.28	0.98	0.03	0.88	0.17
Log-linear [a]	0.97	0.000 4	0.91	0.003	0.99	0.000 1	0.92	0.003	0.89	0.005	0.99	0.000 7
Log-quadratic [b]	0.98	0.000 3	0.98	0.002	0.99	0.000 1	0.98	0.004	0.94	0.01	0.99	0.000 7
Log-cubic [b]	0.98	0.03	0.98	0.02	0.99	0.003	0.98	0.03	0.96	0.06	0.99	0.004

Note: See Zhang et al. (1996) for further detail. Pooled data for both enzyme types (RM_1 and NQ) were used in the analyses.

[a] The assigned value (the ε value) for the diets without added enzymes was 3.2 U / kg diet.

[b] The differences between the first-order (log-linear) and second-order (log-quadratic) parameters and between the first- or second-order and third-order (log-cubic) parameters were not significant ($P > 0.05$).

Table 3. Regression equations describing the linear relationship between the performance of Leghorn chicks and the logarithmic content of enzymes in the diet (experiment 1).

Parameter	Equation [a,b]	Probability	
		Intercept (*A*)	Slope (*B*)
BW gain (g / 6 birds)			
Week 1	$Y = 185 + 29 \log X$	0.000 1	0.000 4
Week 2	$Y = 331 + 16 \log X$	0.000 1	0.003
Weeks 1 + 2	$Y = 517 + 45 \log X$	0.000 1	0.000 1
Feed–gain ratio			
Week 1	$Y = 2.67 - 0.17 \log X$	0.000 1	0.003
Week 2	$Y = 2.33 - 0.04 \log X$	0.000 1	0.005
Weeks 1 + 2	$Y = 2.46 - 0.09 \log X$	0.000 1	0.000 1
General equation	$Y = A + B \log X$		

Note: Data were calculated from pooled data from the two enzymes (RM_1 plus NQ). See Zhang et al. (1996) for further detail. BW, body weight.

[a] The activity value (ε) that was used for the diet without added enzymes was 3.2 U / kg diet. See the text for the derivation of this value. Substitution of this value in the equation will provide an estimate of the performance of chicks with nearly 0 enzymes. The actual values without enzyme addition are given in Table 2.

[b] The equations predict the performance of chicks during week 1, week 2, and weeks 1 + 2 for diets containing different amounts of enzymes (U / kg diet). The variable *Y* is predicted performance (weight gain [g / 6 birds] or feed–gain ratio) of chicks during the different periods; *X* is the amount of enzyme (U / kg diet) added to the diet; *B* is the slope of the line (weight gain [g / 6 birds] or feed–gain ratio per log unit of added enzyme per kilogram diet); and *A* is the intercept of the line (g or g/g). The intercept, *A*, is the estimated performance of chicks fed very low levels of enzyme (the ε value).

where Y is the performance value (for example, weight gain [grams]); A is the intercept (y axis); B is the slope of the line; and X is the concentration of enzyme (grams or units per kilogram of diet). Assume Y_0 = the weight gain over a certain period without added enzyme. Under such conditions, $X = X_0 = 0$, and equation [2] is as follows:

$$Y - Y_0 = A - Y_0 + B \log X \quad [2]$$

Substitute $X + \varepsilon$ for X, as ε is a very small value relative to X. Under such conditions, the r^2 of the equation would not be affected, and equation [3] is

$$Y - Y_0 = A - Y_0 + B \log (X + \varepsilon) \quad [3]$$

When $X = X_0 = 0$, the total amount of enzyme added to the diet is 0. Under such conditions, $Y = Y_0$, and equations [4] and [5] are

$$A - Y_0 + B \log \varepsilon = 0 \quad [4]$$

$$\varepsilon = 10^{(Y_0 - A)/B} \quad [5]$$

The ε value is derived from equation [5] for each set of data and used as a substitute for the 0 value of enzyme concentration. Under such conditions, the ε value that yields the highest r^2 value and the corresponding smallest error (sums of squares) can be calculated. Using these assumptions, I obtained regression equations that predicted weight gains and feed–gain ratios for chicks of different ages (Table 3).

A comparison of the actual performance values obtained in response to the two enzymes (RM_1 and NQ) for weeks 1 and 2 combined and those predicted from the equation demonstrated that in all cases the two sets of values were nearly the same, the maximum difference between them being less than 1% (Figure 1). These differences are relatively small compared with the response obtained with enzyme supplementation. The inset of Figure 1 shows a linear change in weight gain and feed–gain ratio when the concentration of enzyme is plotted on a logarithmic scale. The intercept (A) of the equation represents the weight gain or the feed–gain ratio for a preselected amount of enzyme (the ε value [log 3.2]). The slope of the curve (B), representing weight gain or feed–gain ratio per log unit of enzyme, provides a basis for evaluating the efficacy of an enzyme preparation (see footnotes to Table 3). Data from the slopes of the equation, as shown in Table 3, suggest that the response to enzyme treatment was greater during week 1 than during week 2. The weight gain per log unit of enzyme added per kilogram of feed was 29 g for week 1, 16 g for week 2, and 45 g for weeks 1 and 2 combined (29 g + 16 g). Corresponding B values for the feed–gain ratio were −0.17, −0.04, and −0.09.

General applicability of the model (experiment 2 and data from the literature)

The general applicability of the model was tested using data from a second experiment (Table 4, experiment 2) and from the literature. The objective was to determine whether the log-linear model also yielded high r^2 values with these data when there was a significant response to enzyme treatment. Experiment 2 involved the use of several different concentrations of two enzyme preparations (RM_1 and NQ) having different xylanase activities (Table 4). Linear-contrast comparisons demonstrated that the trends for both preparations were similar to those obtained

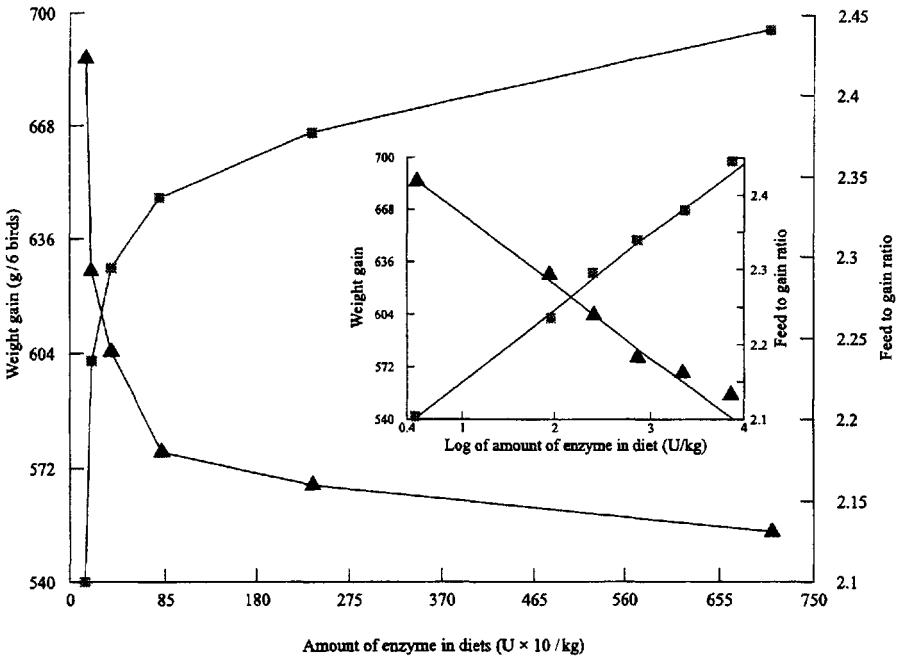

Figure 1. The predicted relationship between chick performance during weeks 1 and 2 of experiment 1 and the amount of crude xylanase added to a rye-based diet, as determined from the equation $Y = 517 + 45 \log X$ or $Y = 2.46 - 0.09 \log X$, where X is units of enzyme in the diet; and Y is weight gain (grams) or the feed–gain ratio (Table 3, experiment 1). Mean experimental values for weight gain (■) and feed–gain ratio (▲) are also shown. Inset figure represents the same data, but the amounts of enzyme have been transformed into their logarithmic values.

in experiment 1. In general, the response to enzyme treatment was greater during week 1 than during week 2 and was not directly proportional to the amount of enzyme added to the diet. For example, the maximum improvement in weight gain obtained during week 1 with added enzyme NQ was 61% ($P < 0.01$) (0 versus 20 U NQ), whereas the corresponding improvement during week 2 was only 17% ($P < 0.01$). Likewise, the addition of a small amount of NQ (1 g of NQ / kg diet) improved weight gains by 42% ($P < 0.01$), whereas the addition of much greater amounts (20 g of NQ / kg diet) produced an additional incremental improvement of only 14% ($P < 0.01$). Similar trends were observed in the feed–gain ratio with NQ and in both weight gain and the feed–gain ratio with RM_1.

Regression analysis of data sets 1–3 (NQ) and 4–6 (RM_1) from Table 4 is outlined in Table 5. The results demonstrate that high r^2 values (0.84–0.999) were obtained during week 1 and weeks 1 and 2 combined, but not during week 2. The slope of the lines (B) shows that the response to enzyme was also high during

Table 4. Performance of Leghorn chicks fed a rye-based diet containing several combinations and concentrations of four crude-enzyme preparations (experiment 2).

		BW gain (g / 6 birds)			Feed–gain ratio		
Diet	Enzyme (g / kg diet)	Week 1	Week 2	Weeks 1 + 2	Week 1	Week 2	Weeks 1 + 2
1	No enzyme (0 U)	174	327	500	2.92	2.16	2.42
2	1 NQ (778 U)	247	344	592	2.35	2.18	2.25
3	20 NQ (15 260 U)	281	384	664	2.18	2.10	2.14
4	0.33 RM_1 (128 U)	210	320	530	2.51	2.23	2.34
5	1 RM_1 (389 U)	231	336	567	2.38	2.25	2.30
6	3 RM_1 (1 167 U)	237	360	597	2.38	2.15	2.24
Pooled SEM		16	22	32	0.13	0.04	0.08
Selected linear contrast (% increase or decrease relative to first comparison)							
1 vs 2 (0 vs NQ)		42**	5	18**	−20**	1	−7**
2 vs 3 (1 NQ vs 20 NQ)		14**	11**	12**	−7*	−4	−5*
1 vs 4 (0 vs 0.33 RM_1)		21**	−2	6	−14**	3	−3
1 vs 5 (0 vs 1 RM_1)		33**	3	13**	−18**	4	−5*
4 vs 5 (0.33 RM_1 vs 1 RM_1)		10**	5	7*	−5	1	−2
4 vs 6 (0.33 RM_1 vs 3 RM_1)		13**	12**	12.7**	−5	−4	−5*
5 vs 6 (1 RM_1 vs 3 RM_1)		2	7	5	0	−5	−3

Note: The average initial weight of chicks in each treatment was 95 g, with 6 birds per replicate. Each treatment consisted of six replicates. The xylanase activities of RM_1 and NQ were 389 and 778 U/g, respectively. Values in parentheses indicate units of xylanase activity per kilogram of diet. BW, body weight; SEM, standard error of the mean.

*,** Significant at $P < 0.05$ and $P < 0.01$, respectively.

week 1 and during weeks 1 and 2 combined, but not during week 2. This response pattern can be attributed to a high degree of response to added enzymes among Leghorn chicks during week 1 but not during week 2. Overall, the data demonstrate that it is possible to predict the response of chicks at different ages to xylanase supplementation, even if the enzyme is from different sources.

Data from the literature were used to determine whether a similar relationship could also be obtained between the log of the amount of enzyme added to the diet and chick performance (data sets 7–19, Table 5). Among the 13 comparisons, 9 yielded r^2 values for weight gain of >0.91, and all but 1 yielded values of >0.77. Regression analysis of the feed–gain ratio yielded similar trends.

In addition, high r^2 values were obtained under a variety of conditions — different supplemented cereals (rye, wheat, and barley) and a grain legume (lupins); different enzyme preparations high in xylanase and β-glucanase activity; different types of enzymes (β-galactosidase, β-glucanase, and xylanase); different proportions of two cereals in the diet (wheat and rye); and different ages and types of chickens (Leghorn and broiler).

Predicting responses to enzyme treatment in chicks fed diets containing various proportions of two cereals

Another objective was to determine whether the simple model equation for predicting chick response to a given diet containing different amounts of one enzyme could be extended to include different proportions of any two dietary components, such as cereals. An experiment in which different amounts of an enzyme were added to diets containing different proportions of two cereals was conducted by Bedford and Classen (1992). They fed four diets with different proportions of rye (0, 20, 40, and 60%), each with six different concentrations of enzyme (xylanase from *Trichoderma longibrachiatum*), to broiler chicks from 1 to 19 d of age in a 4 × 6 factorial arrangement of treatments. The diets contained the following proportions of rye and wheat: 0%, 0 : 60; 20%, 20 : 40; 40%, 40 : 20; and 60%, 60 : 0. Enzyme was added to these diets at 0, 0.1, 0.2, 0.4, 0.8, and 1.6%. The prediction equations derived from equation [1] for weight gain and the feed–gain ratio, obtained from an analysis of the mean data from Bedford and Classen (1992), are summarized in Table 5 for each diet (data sets 8–11).

Multiple regression (SAS 1988) was used to relate the response in chick weight gain (Y) to the enzyme concentration (X) and the proportion of rye (Z) in the diet:

$$Y = B_0 + B_1 \log X + B_2 Z + B_3 Z \log X \qquad [6]$$

Table 5. Prediction of goodness of fit between enzyme concentration

Data set [a,b]	Diet [c] (%)	Enzyme type [d]	Chick type [e]	Age [f] (d)	Weight gain [g]					
							A		*B*	
					r^2	*P*	g [h]	*P*	g/(g/kg) [h]	*P*
1	Rye (60)	Xylanase	L	7–14	0.999	0.01	248	0.002	24.8	0.01
2	Rye (60)	Xylanase	L	14–21	0.81	0.3	358	0.02	11.9	0.29
3	Rye (60)	Xylanase	L	7–21	0.98	0.08	606	0.01	36.8	0.10
4	Rye (60)	Xylanase	L	7–14	0.97	0.02	225	0.000 3	17.4	0.02
5	Rye (60)	Xylanase	L	14–21	0.36	0.4	340	0.000 8	6.5	0.40
6	Rye (60)	Xylanase	L	7–21	0.84	0.08	565	0.000 4	24.2	0.08
7	Rye (60)	Xylanase	L	1–14	0.96	0.000 1	122	0.000 1	29.3	0.000 1
8	Rye (0)	Xylanase	B	1–19	0.49	0.48	436	0.000 1	10.5	0.12
9	Rye (20)	Xylanase	B	1–19	0.92	0.002	420	0.000 1	18.8	0.002
10	Rye (40)	Xylanase	B	1–19	0.94	0.002	419	0.000 1	36.1	0.002
11	Rye (60)	Xylanase	B	1–19	0.96	0.000 5	394	0.000 1	54.4	0.000 5
12	Rye (64)	Xylanase	B	7–21	0.999	0.000 2	123	0.000 1	8.8	0.000 2
13	Wheat (68)	Xylanase	L	7–21	0.92	0.04	132	0.000 1	2.3	0.04
14	Barley (66)	Xylanase	L	7–21	0.96	0.02	133	0.000 1	6.6	0.02
15	Barley (65)	β-glucanase	B	14	0.80	0.2	230	0.002	15.3	0.1
16	Barley (65)	β-glucanase	B	14	0.99	0.006	234	0.000 1	14.0	0.006
17	Barley (65)	β-glucanase	B	21	0.86	0.07	480	0.001	37.4	0.07
18	Barley (65)	β-glucanase	B	21	0.96	0.02	484	0.000 2	32.6	0.02
19	Lupin (50)	β-glactosidase	L	7–21	0.78	0.1	120	0.001	6.8	0.1

[a] Results for data sets 1–6 were obtained from experiment 2. The basal diet contained rye grain supplemented with an enzyme, NQ or RM_1, at different concentrations (units of enzyme activity per kilogram diet): (1) 0 U; (2) 778 U NQ; (3) 15 560 U NQ; (4) 128 U RM_1; (5) 389 U RM_1; and (6) 1 167 U RM_1. The xylanase activities of RM_1 and NQ, as determined by the method of McLeary (1992), were 389 and 778 U/g, respectively. See Zhang et al. for further detail.

[b] Data for data sets 7–19 were from the literature.

[c] The value in parentheses represents the proportion (%) of this constituent in the diet.

[d] For diets 8–11, the concentration of added enzyme in the diet was expressed as a percentage; for all the others, it was expressed in grams per kilogram feed.

[e] L, leghorn; B, broiler.

[f] Age of chicks during the test period.

This model is an extension of the models used previously in this work, in the sense that chick performance is regressed on the logarithm of enzyme concentration. Rye content of the diet (Z) is also accounted for, as is the interaction between rye content and enzyme concentration (the last term in the above model). The response surface for this model is shown in Figure 2. Similar prediction equations can be generated for the feed–gain ratio or any other variable that fits the model.

An important observation from data presented in Figure 2 is that the amount of enzyme required to obtain a given level of performance is much greater when the concentration of the antinutritive factor (that is, arabinoxylan) in the diet is high. For example, the amount of enzyme (percentage of diet) required to obtain

and chick performance as obtained from several different studies.

Feed–gain ratio [g]						
		A		*B*		
r^2	*P*	g/g [h]	*P*	(g/g)/(g/kg) [h]	*P*	Reference
0.99	0.05	2.38	0.007	−0.175	0.05	Exp. 2, diets 1, 2, 3
0.30	0.5	2.14	0.009	−0.010	0.63	Exp. 2, diets 1, 2, 3
0.99	0.06	2.23	0.003	−0.064	0.06	Exp. 2, diets 1, 2, 3
0.97	0.02	2.44	0.000 2	−0.156	0.02	Exp. 2, diets 1, 4, 5, 6
0.07	0.7	2.20	0.000 2	−0.008	0.74	Exp. 2, diets 1, 4, 5, 6
0.93	0.04	2.29	0.000 1	−0.045	0.04	Exp. 2, diets 1, 4, 5, 6
0.94	0.000 3	1.75	0.000 1	−0.496	0.000 3	Friesen et al. (1991)
0.43	0.16	1.50	0.000 1	−0.036	0.16	Bedford and Classen (1992)
0.65	0.05	1.56	0.000 1	−0.052	0.05	Bedford and Classen (1992)
0.62	0.06	1.64	0.000 1	−0.058	0.06	Bedford and Classen (1992)
0.95	0.001	1.62	0.000 1	−0.205	0.001	Bedford and Classen (1992)
0.997	0.002	2.01	0.000 1	−0.090	0.002	Marquardt et al. (1994)
0.99	0.007	1.91	0.000 1	−0.028	0.007	Marquardt et al. (1994)
0.88	0.06	1.83	0.000 1	−0.038	0.06	Marquardt et al. (1994)
0.98	0.01	1.38	0.000 1	−0.041	0.01	Hesselman et al. (1982)
0.93	0.04	1.36	0.000 1	−0.032	0.04	Hesselman et al. (1982)
0.85	0.08	1.74	0.000 4	−0.064	0.08	Hesselman et al. (1982)
0.94	0.03	1.73	0.000 1	−0.054	0.03	Hesselman et al. (1982)
0.92	0.04	2.00	0.000 4	−0.105	0.04	Brenes et al. (1993)

[g] Before regression analysis, the enzyme-activity values were converted to their logarithmic values. In this study, the relationship (r^2) between the log of the concentration of enzyme in the diet and chick performance was determined by regression analysis. The *A* value represents the intercept, and the *B* value represents the slope of the line for the equation $Y = A + B \log X$ (see equation [1] in the text). The ε value was 0.001 g enzyme / kg diet (see equation [5] in the text) and is the value used for the treatment with no added enzyme.

[h] The units for weight gain were grams (*A* value) and grams per gram of added enzyme per kilogram diet (*B* value) for diets 1–7 and 12–19. The concentration of added enzyme in diets 8–11 was expressed as a percentage of the diet, and therefore percentage is used in place of grams of added enzyme per kilogram diet. The corresponding units for the feed–gain ratio were grams per gram (*A* value) and grams per gram per gram of added enzyme per kilogram diet (*B* value). The weight-gain values represent weight gain per six birds for data sets 1–6 and weight gain per bird for all other data sets.

a weight gain of 400 g for chicks fed 20, 40, and 60% rye would be 0.09, 0.51, and 1.07%, respectively, a 12-fold difference between the high and low values. These data were calculated for the equation shown in the caption to Figure 2 but can also be calculated from the parameters given in Table 5, data sets 9–11.

Conclusion

As far as I am aware, no similar model has been used before to predict the enhancement of the nutritive value of different diets achieved by adding enzymes. In this study, the model equation was able to predict the performance response of chicks fed diets containing different amounts of enzyme and different proportions

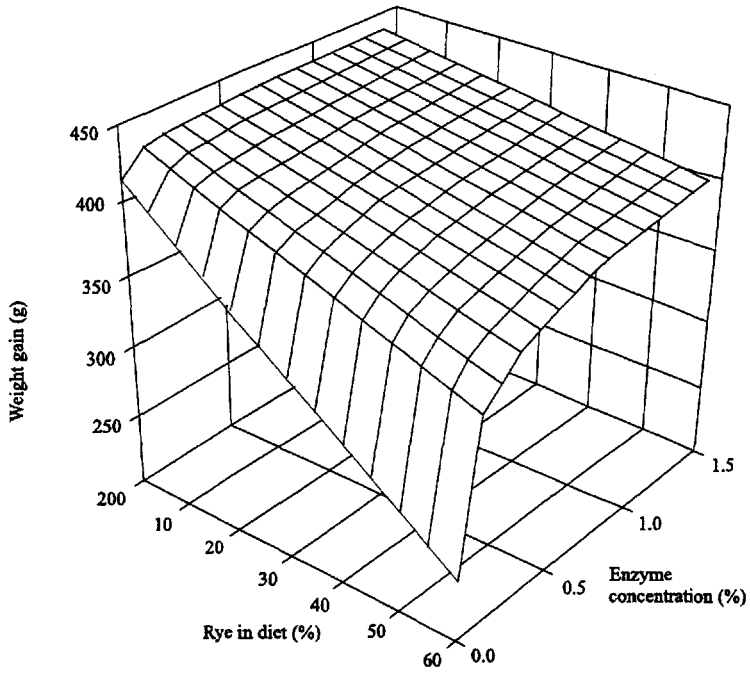

Figure 2. Effect of enzyme concentration (X) and rye content of diet (Z) on chick weight gain (Y): $Y = 436.11 + 7.58 \log X - 0.63Z + 0.75Z \log X$. All coefficients in the equation were significantly different from 0 ($P < 0.001$), with the exception of the coefficient for $\log X$ ($P < 0.1$; $r^2 = 0.94$).

of two cereals. In many of the comparisons, r^2 values of >0.90 were obtained, indicating that the prediction equation is accurate. The equation is also simple, as the improvement in performance with added enzyme is directly related to the logarithmic concentration of the enzyme in the diet. The results demonstrate that for each 10-fold increase in the amount of enzyme, chick performance is improved 2-fold. Under such conditions, a small amount of enzyme may produce a significant improvement, whereas near maximal improvement would require as much as 100- or 1 000-fold more enzyme. The data also suggest that much higher amounts of enzyme may be required for diets with higher amounts of antinutritive factor.

The efficacy of the enzyme can be readily obtained from the slope of the regression equation (B, performance change per log unit of enzyme in the diet) and is influenced by many factors, such as the amount of antinutritive factor in the diet, the age and type of chick, the type of enzyme used, the method of expressing the amount of enzymes added to the diet (that is, units of activity versus an amount per unit weight of diet), and the particular performance criteria measured (weight gain, feed–gain ratio, absorption of nutrient from the diet, etc.). The log-linear model can be used to estimate the efficacy of an enzyme preparation with

respect to its effect on other factors such as nutrient digestion and absorption (unpublished observations). Overall, the model provides a new approach to establishing the efficacy of enzymes added to chicken diets and may apply in the case of other species of animals.

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Phytases in cereals and hemicelluloses in canola (rapeseed) meal and lupins

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As suggested in my earlier paper, "Practical Experiences with the Use of Enzymes" (Guenter, this volume), hydrolysis of fibre or nonstarch polysaccharide (NSP) fractions in cereal grains (wheat, oats, barley, triticale, and rye) has been the focus of most of the research. This area of research is far enough advanced that enzymes are commonly used in poultry diets to enhance the nutritive value of cereals. In recent years, with the development of new enzyme preparations, other target substrates have been investigated. Phytase, which targets the phytate phosphorus in feedstuffs, and α-galactosidases, which enhance the use of protein sources, lupins, and legumes, are gaining attention in research (Campbell and Bedford 1992). Often, these enzymes are used in concert with other enzymes to facilitate more effective hydrolysis of the antinutritive factors in diets.

Phytate

About two thirds of the phosphorus in plant ingredients for pigs and poultry is in the form of salts of phytic acid (myoinositol hexakisphosphates, phytates), which are not very soluble and of very limited digestibility.

The level of phytate phosphorus in feedstuffs generally depends on the part of the plant from which it is derived. Oilseed meals and cereal by-products contain large amounts of phytate phosphorus, whereas cereals and grain legumes contain moderate amounts (Ravindran et al. 1995). Table 1 shows various feedstuffs and the levels of phytate phosphorus present.

The availability of phytate phosphorus to poultry has been determined as ranging from 0 (Nelson 1976) to more than 50% (Edwards 1983, 1993). Ballam et al. (1985) reported hydrolysis values ranging from 3 to 42%. This research and that of others suggested that the availability of phytate phosphorus depends on the level of calcium in the diet, type of ingredient, level of inorganic phosphorus (Temperton and Cassidy 1964), age of the animal, and level of vitamin D_3 (Mohammed et al. 1991; Edwards 1993).

Table 1. Phytate phosphorus content of various feed ingredients.

	Phytate-P (g / 100 g DM)	Phytate-P (as % of total)
Cereals		
Corn	0.24	72
Wheat	0.27	69
Barley	0.27	64
Sorghum	0.24	66
Rice (unpolished)	0.27	77
Grain legumes		
Field peas	0.24	50
Oilseed meals		
Soybean meal	0.39	60
Rapeseed meal	0.70	59
Sunflower meal	0.89	77

Source: Modified from Ravindran et al. (1995).
Note: DM, dry matter.

The general inability of poultry and pigs to utilize phytate phosphorus presents several practical problems:

- The diet also has to be supplemented with inorganic phosphorus (the most expensive mineral);
- Large amounts of phosphorus are excreted in the manure, a pollution problem; and
- Phytate has the ability to bond with other metal ions (for example, Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}), resulting in other nutritional concerns.

For pig and poultry diets, the phytate phosphorus must be hydrolyzed to six inorganic phosphates and inositol. Enzymes capable of hydrolyzing phytates are widely distributed in microorganisms, plants, and animals.

Two phytases are recognized (IUPAC–IUB 1976): 3-phytase (EC2.1.3.8), which hydrolyzes the ester bond on position 3 first, and 6-phytase (EC3.1.3.26), which hydrolyzes phosphate at position 6 first. However, both phytases eventually hydrolyze all six phosphates.

Table 2. Endogenous phytase activity of some feed ingredients.

	Phytase activity (U / kg diet) [a]
Wheat	1 193
Barley	582
Rye	5 130
Corn	15
Rice bran	122
Lupins	0
Soybean meal	8
Rapeseed meal	16

Source: Eeckhout and De Paepe (1994).
[a] 1 μmol/min at pH 5.5, 37°C (substrate Na · phytate).

The degradation of phytate in the digestive tract may be due to intestinal phytase; phytase produced by microorganisms resident in the intestine; and endogenous phytase activity in feed (Table 2). The low levels of phytate phosphorus availability that have been reported indicate that the intestinal phytase and the phytase produced by the intestinal microbes are of little significance, at least in young birds. The endogenous phytase in feeds has long been shown to be partially responsible for the hydrolysis of phytate phosphorus.

Unfortunately, the optimum pH for endogenous phytase activity is in the range of 4.0–6.0 (Irving 1980), although some activity may be retained at pH 3.0. Table 3 shows the phytase activity in corn and wheat at various pH levels. Significant amounts of enzymes are highly unlikely to survive the highly acidic conditions (pH 1.0–2.5) of the proventriculus and gizzard (Hill 1971), where the solubility of phytate is also high (Von Sheuermann et al. 1988). Endogenous phytase is also heat labile and is readily inactivated at 70–80°C, the temperatures commonly used in feed processing (Jongbloed and Kemme 1990). Therefore, to improve the utilization of phytate from feedstuffs, extensive research has been conducted on the use of microbial phytase, which is active at a broader range of pH (2.5–5.5) and is more heat resistant (Table 4).

Although the low supply and high costs of microbial phytase (Han 1989) limited its commercial exploitation in the past, the advancement of biotechnology has generated new interest, particularly in areas where laws stipulate that the excretion of phosphorus be reduced.

Table 3. Influence of pH on phytate solubility and phytase activity in corn and wheat.

	Phytate solubility (%)		
pH	Corn	Wheat	Phytase activity [a]
<1.0	100	100	Inactive
1.0	75	80	Inactive
2.0 and 3.0	38	45	Inactive (?)
4.0	52	58	Active
5.0	82	60	Active
6.0	90	70	Active
7.0	95	80	Inactive (?)
8.0	93	73	Inactive

Source: Von Sheuermann et al. (1988).
[a] Expected activity based on pH ranges; (?) indicates uncertainty.

Table 4. Effect of pelleting temperature on the survival of phytase.

	Pelleting temperature (°C)	Phytase activity (U/kg)	Remaining activity (%)
Feed enzyme before pelleting		250	100
Meal temperature before pelleting (°C)			
50	78	240	96
50	81	234	94
65	84	208	83
65	87	115	46

Source: Simons et al. (1990).

Microbial phytase as a feed additive

Almost three decades ago, Nelson et al. (1968) were the first to show that the phytate phosphorus in diet ingredients like soybean meal could be hydrolyzed by a phytase produced by *Aspergillus ficuum*. The following year, Rojas and Scott (1969) reported that phytase from *A. ficuum* almost completely hydrolyzed the phytate in cottonseed meal.

The upsurge of interest in phytase of microbial origin is made evident by the increasing number of publications (see Simons et al. 1990; Schöner et al. 1991; Jongbloed et al. 1992; Kornegay 1996; Qian et al. 1996; Yi et al. 1996) and presentations of data on this topic at scientific meetings and nutrition symposia (Wenk and Boessinger 1993; Ravindran et al. 1995; BASF 1996).

Table 5. Performance of piglets fed barley–wheat–soy diets supplemented with inorganic phosphorus or phytase.

Total dietary P (%)		Phytase activity	ADG	Feed conversion	Feed–gain ratio
Starter	Grower	(U/kg)	(g/d)	(kg/d)	
0.6	0.48	—	769*a*	1.59*a*	1.99*a*
0.5	0.38	—	703*a*	1.45*a*	2.06*b*
0.5	0.38	500	739*a*	1.47*a*	1.98*a*
0.45	0.35	750	759*a*	1.53*a*	2.02*ab*

Source: Modified from Schöner et al. (1993).
Note: ADG, average daily gain.
a,b, Means within a column not followed by the same letter are significantly different ($P < 0.05$).

The response to using phytase in diets with low levels of available phosphorus has been uniformly positive for both pigs and poultry. Schöner et al. (1993) reported on a study of piglets fed a barley–wheat–soy diet supplemented with either inorganic phosphorus or phytase (Table 5). Their data demonstrated that a diet with low phosphorus content (0.5% in the starter diet and 0.38% in the grower diet) but supplemented with phytase resulted in growth rates and feed-conversion efficiency similar to those obtained with diets supplemented with a higher concentration of inorganic phosphorus (0.6% in the starter diet and 0.48% in the grower diet).

Similarly, Jongbloed et al. (1993) demonstrated that adding phytase at 1 500 U/kg basal diet resulted in better piglet performance than adding 0.1 or 0.2% inorganic phosphorus (Table 6). The data suggest that 1 500 U phytase/kg diet significantly increases the availability of phytate phosphorus.

Table 6. Performance of piglets (11–30 kg BW) when fed a basal diet supplemented with inorganic phosphorus (0.1 or 0.2%) or phytase (1 500 U/kg diet).

Treatment	*n*	BW (kg)	ADG (g)	Feed intake (g/d)	Feed–gain ratio
BD	93	23.5*a*	424*a*	695*a*	1.65*a*
2BD + 1 g P_i	95	24.7*b*	469*b*	739*b*	1.59*b*
BD + 2 g P_i	95	24.4*ab*	456*b*	735*b*	1.62*ab*
BD + phytase	93	26.5*c*	529*c*	802*c*	1.52*c*

Source: Jongbloed et al. (1993).
Note: ADG, average daily gain; BD, basal diet; BW, body weight; P_i, inorganic phosphorus.
a–c, Means within a column not followed by the same letter are significantly different ($P < 0.05$).

Table 7. Effect of microbial (*Aspergillus*) phytase on the availability of phosphorus and the performance of broilers (0–4 weeks).

Total P (%)	Added phytase (U / kg diet)	P availability (%)	Growth (g)	Feed–gain ratio
0.45	0	51.6*a*	788*a*	1.59
0.60	0	46.2*b*	1 066*b*	1.58
0.75	0	41.4*c*	1 081*b*	1.59
0.45	375	60.0*d*	1 101*b*	1.57
0.45	750	61.7*d*	1 087*b*	1.58
0.45	1 500	62.3*d*	1 139*b*	1.54
0.45	2 000	62.6*d*	1 125*b*	1.56

Source: Modified from Simons et al. (1990).
a–d, Means within a column not followed by the same letter are significantly different ($P < 0.05$).

Cromwell et al. (1993) fed pigs growing–finishing diets supplemented with 250, 500, or 1 000 U microbial phytase / kg diet. Linear increases in growth rate, feed intake, and feed–gain ratios were observed. The 1 000-U dose converted about one third of the unavailable phosphorus to an available form. It was estimated that 500 U phytase / kg diet generated a level equivalent to 1 g of inorganic phosphorus.

Similar beneficial results have been reported for poultry. Simons et al. (1990) evaluated a low-phosphorus (0.45%) diet supplemented with inorganic phosphorus (0.15 or 0.3%) or microbial phytase (375, 750, 1 500, or 2 000 U / kg diet) (Table 7). The lowest level of phytase resulted in a significant increase in the availability of phosphorus. Although the two highest levels of phytase slightly increased the availability of phosphorus and the growth rate, these increases were not significant. The addition of 375 U phytase / kg diet was as effective as adding 0.15 or 0.3% inorganic phosphorus. Cromwell (1991) demonstrated a significant reduction in the dietary phosphorus required and manure phosphorus excreted when phytase was added to a diet. This reduced environmental pollution.

More recently, a study of the effectiveness of adding phytase to chick diets low in available phosphorus was conducted in our laboratory (B.A. Slominski and W. Guenter, unpublished). Broiler chicks were fed a wheat–canola-based diet with three levels of available phosphorus (0.2, 0.3, and 0.4%) and four levels of added microbial phytases (0, 100, 200, and 400 U / kg diet). The data (Table 8) clearly demonstrate that weight gain and feed–gain ratio improved with the addition of inorganic phosphorus. Similarly, the lowest level of added phytase resulted in a significant improvement in weight gain and feed–gain ratio. Higher levels of added phytase produced no additional environmental responses.

Table 8. Effect of phytase supplementation on the availability of phosphorus in a wheat–canola-meal-based diet for broiler chicks.

Available P (%)	Phytase level (U / kg diet)				
	0	100	200	400	$\bar{X}$
	0- to 2-week weight gain (g)				
0.2	82.0	87.6	90.5	87.7	86.9*c*
0.3	86.8	89.2	92.1	95.5	90.9*b*
0.4	89.7	98.2	96.3	95.7	95.0*a*
$\bar{X}$	86.2*b*	91.7*a*	92.9*a*	93.0*a*	93.0*a*
	0- to 2-week feed–gain ratio				
0.2	2.31	2.22	2.20	2.22	2.24*a*
0.3	2.27	2.22	2.24	2.23	2.24*a*
0.4	2.24	2.16	2.16	2.15	2.18*b*
$\bar{X}$	2.27*a*	2.20*b*	2.20*b*	2.20*b*	2.20*b*

Source: B.A. Slominski and W. Guenter (unpublished).
a–c, Means within a row or column not followed by the same letter are significantly different ($P < 0.05$).

In a further study, Simbaya et al. (1996) demonstrated that the effectiveness of microbial phytase could be enhanced by adding protease and carbohydrase to chick diets (Table 9). Although a slight response was observed when 0.01% phytase was added to a diet containing 0.35% available phosphorus, this response was greatly enhanced by the addition of 0.05% each of a protease and a carbohydrase preparation — performance was equivalent to that obtained with the control diet that had 0.45% available phosphorus. The data suggest that these enzymes had a synergistic effect on phytase activity.

In a recent publication (BASF 1996), the current recommendations for the use of microbial phytase were summarized for pigs and poultry as follows:

- All classes of pigs, 500 U / kg diet;
- Laying hens, 300 U / kg diet; and
- All other chickens, 600 U / kg diet.

These phytase levels will reduce the inorganic phosphorus requirement by 1 g / kg diet and the calcium requirement by a similar amount, except in the case of laying hens, for which the calcium requirement is reduced by 3 g / kg diet.

Table 9. Synergistic effects of enzyme supplements on the performance of broiler chicks (4–11 d of age) fed practical wheat–canola-meal-based diets.

Diet–enzyme [a]	Feed intake (g)	Weight gain (g)	Feed–gain ratio
Control (0.35% P_a)	177	116.7*b*	1.52*a*
+ Pht	175	118.0*ab*	1.48*ab*
+ Pht, Prt	171	116.5*b*	1.47*ab*
+ Pht, Carb	171	117.0*b*	1.46*ab*
+ Pht, Prt, Carb	176	124.2*a*	1.41*b*
Control (0.45% P_a)	176	124.8*a*	1.42*b*

Source: Simbaya et al. (1996).

Note: Carb, carbohydrase; P_a, available phosphorus; Pht, phytase; Prt, protease.

[a] Enzyme inclusion rates for phytase, protease, and carbohydrase were 0.01, 0.01, and 0.05%, respectively.

a,b, Means within a column not followed by the same letter are significantly different ($P < 0.05$).

Hemicelluloses in canola (rapeseed) meal and lupins

Hemicelluloses are components of the polysaccharide fraction of the cell wall in higher plants. Hemicelluloses are associated with cellulose and pectic substances and comprise several nonstarch, noncellulosic polysaccharides, including xylans (arabinoxylans and 4-0 methyl glucuronoxylans), galactomannans, glucomannans, β-D-glucans (3- and 4-linked), β-D-glucan-callose (3-linked), and xyloglucans (4-linked β-D-glucans with attached side chains) (Chesson 1987). The β-glucans and arabinoxylans have been recognized as being the antinutritive factors in cereals (Bedford 1995). Early work identified the soluble β-glucans and arabinoxylans as the fractions responsible for causing a viscous intestinal environment and thus for impeding digestion (Antoniou and Marquardt 1982; White et al. 1983). The effect of these soluble NSPs on nutrient availability and animal performance is extensively discussed in this volume.

Canola meal

Canola (rapeseed) meal, a by-product of the canola-oil industry, is recognized as a good protein source for animal feeding (Bell 1982). In general, the amino acid pattern resembles that of the ideal protein proposed for pigs (ARC 1981) and poultry (Baker and Chung 1992). High arginine and sulfur amino acid contents make the meal especially valuable for poultry.

The carbohydrate component of canola meal accounts for about one third of the meal and is composed of sucrose, oligosaccharides, starch polysaccharides,

Table 10. Carbohydrate content of brown- and yellow-seeded canola meal and soybean meal.

	Carbohydrate content (% DM)		
	Canola meal		Soybean meal
	Brown	Yellow	
Glucose and fructose	0.5	0.6	0.5
Sucrose	7.7	9.8	6.9
Oligosaccharides	2.5	2.4	5.3
Starch polysaccharides	2.5	2.6	0.7
NSPs	17.9	21.4	20.3
Cellulose	4.6	6.0	5.5
Hemicellulose–pectin	13.3	15.4	14.8
Lignin and polyphenols	8.0	3.2	1.0
Total carbohydrates	31.1	36.8	33.7

Source: Slominski and Campbell (1990, 1991).
Note: DM, dry matter; NSP, nonstarch polysaccharide.

and NSPs (Table 10). The carbohydrates with low molecular weights are similar to those of soybean meal; however, soybean meal contains higher levels of oligosaccharides. Because of the high fibre content in canola meal, the energy level for poultry is very low (7 950–8 370 kJ/kg), about 1 170 kJ/kg lower than that available in soybean meal (Clandinin 1990).

As noted earlier, the solubility of the NSPs in cereals is a major contributor to poor performance. The NSPs in canola have a relatively low water solubility (Slominski et al. 1994) and therefore may not respond to enzyme supplements the way cereals do.

In a laying-hen study using a semipurified diet with 40% canola meal, with and without a crude-enzyme (high in polygalacturonase) preparation (Slominski and Campbell 1990), the enzyme supplement increased the digestion of noncellulose polysaccharides from 3.2% to 40.5%. However, the benefits of this increased digestion were not demonstrated.

Numerous protease and carbohydrase preparations have been tested with canola meal, some showing promise (Table 11). Carbohydrase 2 resulted in a 5% improvement in feed–gain ratio and a trend toward improved growth. Simbaya et al. (1996) showed the synergistic effects of protease, carbohydrase, and phytase when fed to chicks 4–11 d of age (see Table 9). By week 2, however, this significant effect appeared only as a trend. The data suggest the potential for developing an effective enzyme "cocktail" for canola-meal diets, but further research is required.

Table 11. Performance of broiler chicks fed semipurified canola-meal diets with carbohydrase supplementation.

Enzyme	Feed intake (g)	Weight gain (g)	Feed–gain ratio
None	540	352	1.53*a*
Carbohydrase 1 (0.05%)	544	362	1.50*ab*
Carbohydrase 2 (0.05%)	559	382	1.46*b* [a]
Carbohydrase 3 (0.05%)	544	357	1.52*a*

[a] 5% improvement.

a,b, Means within a column not followed by the same letter are significantly different ($P < 0.05$).

The oligosaccharides raffinose, stachyose, and verbascose — which account for 2.5% of canola meal (Slominski and Campbell 1991) — have been implicated in a depressing effect on the utilization of energy from soybean meal in poultry (Coon et al. 1990). These α-galactosides cannot be hydrolyzed in the small intestine of monogastric animals because of the absence of α-(1,6)-galactosidase (Irish et al. 1995). High concentrations of these oligosaccharides in the alimentary tract may result in fluid retention and an increased flow rate of digesta, which adversely affects utilization and absorption of nutrients. These compounds are not broken down in the small intestine but ferment in the hind gut, resulting in flatulence and disruption in digestion (Classen 1996).

Coon et al. (1990), Leske et al. (1991), and Leske et al. (1993a) reported that using ethanol to extract α-galactosides from soybean meal resulted in improvements in NSP digestion and true metabolizable energy (TME_N). Adding stachyose and raffinose to a soy-protein concentrate resulted in reduced TME_N (Leske et al. 1993b). Irish et al. (1995) found that ethanol extraction resulted in an increase in the gross energy of soybean meal but not in its TME_N. Slominski et al. (1994) found that ethanol extraction in the case of canola meal was not beneficial and resulted in decreases in gross energy and TME_N. These effects were attributed to the fact that canola meal contains more ethanol-soluble substances but has lower levels of oligosaccharides than soybean meal. Coon et al. (1990) also reported improved fibre digestion after ethanol extraction; however, Slominski et al. (1994) found that removing oligosaccharides from canola meal with dietary enzymes did not alter NSP digestibility and concluded that ethanol-soluble components other than oligosaccharides were responsible for the improvements in fibre digestion and TME_N when soybean meal was subjected to ethanol extraction. This agrees with the findings of Angel et al. (1988) and Irish et al. (1995).

Table 12. Performance of broiler chicks (7–21 d of age) fed enzyme-supplemented 70% lupin-seed diets.

Enzyme	Feed intake (g)	Weight gain (g)	Feed–gain ratio
None	630	367*c*	1.72*a*
EN (0.1%)	638	394*abc*	1.62*abc*
BP (0.1%)	670	416*ab*	1.61*bc*
NOV (0.1%)	637	386*bc*	1.65*ab*
Cellulase TV (0.1%)	647	377*bc*	1.72*a*
EN + BP	669	418*ab*	1.60*bc*
EN + NOV	674	414*ab*	1.63*abc*
BP + NOV	628	404*abc*	1.56*bc*
EN + BP + NOV	671	433*a*	1.55*c*

Source: Brenes et al. (1993).
Note: BP, Bio-Feed Pro®; NOV, Novozyme®; EN, Energex®
a–c, Means within a column not followed by the same letter are significantly different ($P < 0.05$).

Lupins

Aguilera et al. (1985) reported that the hemicellulose fraction of lupin seed was much smaller than that of barley (3.4 versus 13.6%, respectively). Similarly, Batterham et al. (1986) reported very low levels of hemicellulose in lupin-seed meal (1.7 and 2.7%). In contrast, Cheung (1991) (cited by Annison and Choct [1993]) reported that the oligosaccharide fraction in lupins was 8–9%. This high level of α-galactosides may have an antinutritive effect on chicks fed lupin-containing diets.

Brenes et al. (1993) reported studies evaluating the effects of several enzyme preparations on the nutritive value of lupins for chicks. Four enzyme preparations were used: Energex®, high in hemicellulase, β-glucanase, and pectinase activities; Bio-Feed Pro®, high in protease activity; Novozyme SP230®, high in α-galactosidase and inulinase activities; and Cellulase TV, high in cellulase activity. The enzyme preparations were incorporated alone or in combination, each at a level of 0.1%, in a 70% lupin diet. Although the carbohydrases (Energex) and the α-galactosidases (Novozyme SP230) gave a slight improvement in growth rate and feed efficiency, these responses were not significant. Adding cellulase was of no benefit (Table 12).

A significant ($P < 0.05$) response was observed, however, when the preparation with high protein-hydrolyzing activity (Bio-Feed Pro) was added to the diet, alone or in combination with Energex or Novozyme. The greatest responses (18% increase in weight gain and 10% improvement in feed–gain ratio) were observed

from the combination of all three enzyme preparations, suggesting a synergistic effect.

Carre and Leclercq (1985) showed that the cell-wall material in lupin cotyledons is composed of a high concentration of pectin-like substances and low concentrations of cellulose and lignin. The pectin-like substances are composed of branched β-(1→4)-galactans, which should be highly fermentable (Carre et al. 1985) and therefore may not be the principal antinutritive factor in lupins. Brenes et al. (1989) showed that diets containing oligosaccharide extract (dried) from lupin seeds did not impair chick performance. However, these compounds may be the target of α-galactosidases, and the synergistic effect demonstrated by Brenes et al. (1993) suggests that α-galactosidases may work in concert with other enzymes to improve the nutritional value of raw lupin seeds.

Supplementing diets with enzymes not only improved the nutritional value of lupins but also reduced the length and size of various sections of the gastrointestinal tract and the size of the pancreas, suggesting a decreased microbial population due to a reduction in intact complex carbohydrates.

Conclusions

1. Adding 300–600 U of phytase to the diets of pigs and poultry was equivalent to adding 0.1% of inorganic phosphorus.

2. The performance of chicks fed a canola-meal diet was improved to the greatest degree when phytase was added to the diet in combination with a protease and a carbohydrase.

3. Oligosaccharides did not appear to be major antinutritive components of canola meal and lupins.

4. Lupins and canola meal both responded best to a complex enzyme cocktail.

5. Further work is required to develop the best enzyme combinations for diets containing canola meal and lupins.

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Exogenous enzymes for pig diets: an overview

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To achieve high production efficiency in the pig industry at low cost, a continuous improvement in the utilization of diets and of a wide range of dietary ingredients is crucial. Modern pig producers use cereal feeds in combination with certain by-products, and profitability highly depends on the relative cost and nutritive value of the selected feedstuffs. For these reasons, a continuous effort has been made to understand the complex nature of feed components. The most promising development has been the use of certain microbial enzymes to increase the nutrient availability of cell-wall carbohydrates. One of the problems in the pig industry is the use of drugs like antibiotics and growth promoters, which have an adverse impact on human health and the environment. Substitutions for these chemically synthesized drugs must be harmless, environmental friendly, and "natural." Enzymes may be able to meet these requirements.

Biochemically, enzymes are proteins consisting of individual amino acids, but they may also contain other substances or cofactors, such as vitamins and minerals. Commercially available feed enzymes are natural products, produced by microbial fermentation. Enzymes, as biological catalysts, are involved in all anabolic and catabolic pathways of digestion and metabolism. On the one hand, they enable the pathways to operate efficiently under the metabolic conditions. On the other hand, they act as regulators of individual processes. Because of these characteristics, interest is growing in the use of enzymes to improve animal performance. Numerous successful studies on the supplementation of poultry diets with enzymes have been reviewed by several authors (Campbell and Bedford 1992; Walsh et al. 1993; Bedford 1995; Dierick and Decuypere 1996), but information on pigs seems to be inconclusive. This paper will discuss potential benefits of, and problems with, the use of the enzymes in pig diets.

Enzymes for pigs — theoretical assessment and practical significance

In a highly developed animal, nutrients like proteins, fat, and carbohydrates can only be absorbed in nutritionally significant quantities in the form of free amino

acids, fatty acids, and monosaccharides, or oligopeptides–saccharides. Such digestive processes will not occur without the relevant enzymatic reactions. The endogenous enzymes produced by animals may be insufficient in special circumstances. Supplementation of diets with exogenous enzymes would therefore help the animal cope in these situations.

Weaning

The change in environment at weaning often remarkably challenges an animal's ability to secrete endogenous enzymes. Before piglets can satisfactorily cope with the postweaning diet, therefore, they may need a period of adaptation to increase the physical size of the gastrointestinal tract; its capacity to secrete digestive enzymes, HCl, bicarbonates, and other chemicals; and its absorptive capacity. Linderman et al. (1986) reported that the total amount of enzymes secreted and the amounts per gram of pancreas increased linearly in the pig after birth. Weaning at 28 d of age caused a sudden drop in the activities of amylase, protease, and lipase the following week. This resulted in severe digestion problems, including reduced nutrient absorption and diarrhea. Adding the appropriate enzymes to the diet, in combination with an optimal feed composition, should overcome such problems. (The activities of the endogenous enzymes generally return to normal levels over several weeks.)

Energy availability

Many feed ingredients have characteristics unfavourable to the digestive system of the pig. The best known example is nonstarch polysaccharides (NSPs) in the cell walls of grains. The pig is unable to release the energy portion of such components, as this complex carbohydrate is resistant to the pig's endogenous enzymes. However, microbial enzymes added to the diet are effective against certain growth-depressing components, such as β-glucan (Inborr and Ogle 1988). Some feed ingredients that are considered alternative feedstuffs in the grain-dominated countries are commonly used in many developing countries. Because these feedstuffs often contain certain growth-depressing factors, enzyme supplementation is beneficial. For example, enzymes added to linseed-meal diets (an unconventional ingredient) improved pig performance (Officer and Butterham 1992). In contrast, most conventional ingredients are highly digestible, particularly those used in creep and starter diets. Table 1 shows how piglet performance is affected by exogenous enzymes in diets containing proteins of vegetable and animal origin.

Enhanced energy availability may also be achieved by shifting the site of digestion from the large to the small intestine. Significant hindgut fermentation may degrade the cell walls to a large extent; and in the large intestine only about

Table 1. Performance of piglets fed enzyme-supplemented diets containing animal or vegetable protein from weaning (25 d) to 46 d.

	Vegetable protein		Animal protein	
	Enzyme	Control	Enzyme	Control
Daily gain (g)	320	300	310	310
Feed intake (g/d)	320	300	440	460
Feed–gain ratio	1.32*a*	1.63*c*	1.42*b*	1.53*bc*

Source: Modified from Johnson et al. (1993).
a–c, Means within a row not followed by the same letter are significantly different ($P < 0.05$).

Table 2. Nutrient disappearance in the small and large intestines of finisher pigs fed barley-based diets with or without added enzymes.

		Nutrient disappearance (%)	
Digestion site	Treatment	Energy	Nitrogen
Ileum	Control	61.2	63.7
	Enzyme	77.8	76.9
Large intestine	Control	17.3	11.0
	Enzyme	0.3	−0.2
Overall	Control	78.4	74.7
	Enzyme	78.4	76.7

Source: Taverner and Campbell (1988).

30% of the derived energy may be utilized by the animal, and this is in the form of volatile fatty acids. However, the degradation and absorption of dietary fibre in the small intestine may be limited by the lack of appropriate enzymes. Theoretically, exogenous enzymes can enhance this degradation. Taverner and Campbell (1988) showed that an enzyme preparation containing β-glucanase increased the availability of dietary energy by 13% and increased dietary-protein absorption by 21% in pigs fed barley-based diets (Table 2). This was achieved by altering the site of digestion from the large to the small intestine.

Pollution control

In recent years, environmental deterioration resulting from animal husbandry has become a concern in both developed and developing countries. The main issue is nitrogen and phosphorus in animal excreta. Excessive nitrogen yields ammonia, which pollutes the air, and bacteria will convert the nitrogen into nitrate, a compound that can contaminate soil and water. About two thirds of the phosphorus in plants is bound to phytate, and this is almost indigestible in monogastric animals

because they lack the endogenous phytase to liberate phosphorus from phytate. This undigested phosphorus contributes to phosphorus pollution, especially in areas of intensive livestock production. Inorganic phosphorus is therefore routinely added to diets to satisfy the animal requirements for this nutrient. This supplement also takes up space in the formulation that could be used by cheaper raw ingredients. Simons et al. (1990) and Jongbloed et al. (1993) clearly demonstrated that adding microbial phytase to diets deficient in available phosphorus enhanced the utilization of phosphorus. Moreover, significant improvement in growth can be achieved at low levels of available phosphorus (Jongbloed et al. 1993). When legislation on manure disposal comes into effect, interest in using microbial enzymes will increase.

Enzyme efficacy — an overview of the published studies

Numerous studies have, to a large extent, confirmed the biological efficacy of microbial phytase in improving the pig's utilization of phosphorus from phytate. Microbial phytase has been the most successful example of exogenous enzymes used in pig diets (Johnson et al. 1993).

Although enzyme manufacturers provide plenty of data showing positive responses to carbohydrate-degrading enzymes, experimental results published in the scientific literature vary — both positive and nil effects can be found. Summaries of some typical studies are given in Tables 3 and 4.

Digestibility

Several studies have investigated the effects of enzyme supplements on nutrient digestibility in pigs (see Table 3). Dierick and Decuypere (1996) reported improvements in both *in vitro* and *in vivo* digestibilities. With wheat-by-product diets, ileal digestibilities of protein, fat, NSPs, dietary fibre, amino acids, and phosphorus increased substantially. However, these enhancements were not accompanied by relevant incremental improvements in dry- and organic-matter digestibilities. In contrast, a series of digestibility studies recently conducted in our laboratory showed a consistent increase in ileal digestibility of dry matter, gross energy, and amino acids (Y-G. Liu and S.K. Baidoo, unpublished). The majority of the digestibility studies in Table 3 showed positive responses to added enzymes, with the most significant improvements occurring when the diets were high in β-glucans. Graham et al. (1988) and Inborr et al. (1993) reported 19 and 40% increases in β-glucan digestibilities, respectively. This is consistent with the 6% increase in fecal crude-fibre digestibility reported in the early study of Suga et al. (1978). Wenk et al. (1993) used ground whole maize plants (17% crude fibre and

Table 3. Enzyme efficacy: digestibility studies.

Enzymes	Diet	Response (%) [a]	Reference
Cellulase	Wheat–maize–fish–soya–rice bran	Fecal: CP, 2; CF, 6; fat, 2	Suga et al. (1978)
β-glucanase–xylanase	Barley–pollard–soya	Ileal: β-glucan, 19; CP, 6; ash, 10; fat, 5.5	Graham et al. (1988)
β-glucanase	Barley–soya	Ileal: GE, 2.7; St., 2.7; DF, 1.9 Fecal: GE, 1.4; ash, 25; DF, 1.4	Graham et al. (1989)
β-glucanase–cellulase–xylanase–amylase	Wheat–barley–soya–whey	Fecal: CP, ash, and OM, 4 (4% N retention)	Inborr and Graham (1991)
Xylanase–amylase–pectinase–β-glucanase	Wheat–soya–fish Barley–soya–fish	Fecal: NDF, −3 to −9; fat, −6 to −7	Mellange et al. (1992)
Xylanase–β-glucanase	Rye–soya Barley–soya	Nil effect	Bedford et al. (1992)
Amylase–xylanase–pectinase	Wheat–hypro soya Barley–wheat–soya	Ileal: GE, 4–5; Fecal: GE, 2; β-glucan, 40; soluble NSP, 21–27; St., 3	Inborr et al. (1993)
Carbohydrase	Whole maize plant	Fecal: NDF, 10; ADF, 11; CP, 5; GE, 3.6	Wenk et al. (1993)
Cellulase	Wheat by-product	Ileal: CP, 5.9; NSP, 16.7; fat, 8.2; P, 6.9; DF, 13.7; AA, 4.4–9.4	Dierick and Decuypere (1996)
Cellulase–hemicellulase–protease cocktail	Hull-less barley Hull-less barley–canola	Ileal: DM, 6; GE, 9; CP, 9; AA, 9–15; Ileal: AA, 2–3	Y-G. Liu and S.K. Baidoo (unpublished)

[a] AA, amino acids; CF, crude fibre; CP, crude protein; DF, dietary fibre; DM, dry matter; GE, gross energy; N, nitrogen; NDF, neutral detergent fibre; NSP, nonstarch polysaccharides; OM, organic matter; P, phosphorus; St., starch. Value after abbreviation represents percentage response to enzyme treatment.

Table 4. Enzyme efficacy: growth performance.

Enzymes	Diet	Response (%) [a]	Reference
Cellulase	Wheat–maize–fish–soya–rice bran	DG, 45; FCR, 9	Suga et al. (1978)
β-glucanase	Barley–concentrate	DG, 5; FCR, 5	Thomke et al. (1980)
Amylase–sucrase–amylase–β-glucanase	Maize–soya Cooked barley enzyme	Nil Nil, less diarrhea	Hogberg et al. (1983) Inborr and Ogle (1988)
Amylase–cellulase–β-glucanase–protease	Cooked barley–steamed oats–soya–fish	DG, −8	Inborr et al. (1988)
Cellulase, cellulase–amylase	Maize–soya–ensiled rice bran	Nil for DG; FCR, −32 to −40	Tangendjaja et al. (1988)
Cellulase–β-glucanase Xylanase–amylase	Enzyme-pretreated barley Barley–wheat	a. Nil for DG and FCR b. FCR, 10–15; less diarrhea	Bohme (1990)
Pentosanase β-glucanase	Rye–soya–SMP Barley–soya–SMP	Nil DG, 17; nil for FI	Inborr and Graham (1991)
Xylanase–amylase–pectinase–β-glucanase	Wheat–soya–fish Barley–soya–fish	FCR, 4; nil for DG and FI	Mellange et al. (1992)
Xylanase β-glucanase	Rye–soya Barley–soya	Nil for DG and FCR DG, 17	Bedford et al. (1992)
β-glucanase β-glucanase–xylanase	Barley–soya Barley–wheat–soya	Nil for DG and FI Nil for DG and FCR	Thacker et al. (1992) Inborr et al. (1993)
β-glucanase Xylanase	Barley–soya Wheat–soya	DG, 11.3; FI, 11.5; nil for FCR DG, 6.9; FCR, 6.3; nil for FI	Cos et al. (1993)
Protease–amylase–lipase–β-glucanase	Wheat–fish–meat–tallow–soya–blood meal	Nil for DG and FCR	Officer (1995)
Cellulase–hemicellulase	Hull-less barley–canola	FCR, 3–10; nil for DG	S.K. Baidoo and Y-G. Liu (unpublished)
Enzyme mixture	Hull-less barley	DG, 5–10; FCR, 5–10	Y-G. Liu and S.K. Baidoo (unpublished)

[a] DG, daily gain; FCR, feed-conversion rate; FI, feed intake. Value after abbreviation represents percentage response to enzyme treatment.

42% neutral detergent fibre [NDF]) to replace 50% of the basal diets for pigs weighing about 40 kg. The diets were supplemented with two fungal enzyme preparations. In all cases, enzyme supplementation increased the digestibility of gross energy, nitrogen, NDF, and acid detergent fibre, with improvements as high as 3.6, 5, 10, and 11%, respectively ($P < 0.05$). An interesting finding, apart from the enhanced digestibility coefficients, was that enzyme supplementation reduced the content of the fibre components in the diet by 7%. This may have been due to enzymatic interaction during mixing, storage, or analytical processing.

Our recent study using hull-less barley showed that the response to enzyme supplementation depended on the composition of the diet and the choice of enzymes. The supplementation of hull-less barley with carbohydrate-degrading enzymes significantly increased the digestibility of dry matter, gross energy, and crude protein in the ileum by 8.6, 9.1, and 7.6%, respectively ($P < 0.05$). Fecal protein digestibility increased by 4.7%, although energy digestibility was unchanged. The average ileal digestibility of the essential amino acids in hull-less barley improved by 6.6% with an enzyme preparation high in hemicellulase, whereas the increase obtained using an enzyme high in cellulase was 12% (Table 5). The improvement in the ileal digestibility of lysine, methionine, threonine, and valine was considerable, ranging from about 9 to 15%. The greatest response was obtained using an enzyme preparation high in cellulase activity. On the other hand, no positive responses were observed for the same enzyme preparations added to diets of hull-less barley and canola meal (Y-G. Liu and S.K. Baidoo, unpublished). In this study, enzyme supplementation reduced the viscosity of ileal digesta. Such a reduction is believed to facilitate digestion in the ileum. The studies described above also indicated that the carbohydrate-degrading enzymes improved the digestion not only of NSPs but also of protein, as well as other dietary components, such as fatty acids. The available data indicated that young pigs have a more pronounced response to enzyme supplementation than older pigs and that the type of enzyme and the composition of the diet greatly influence the magnitude of this response. Some studies showed no response in digestibility (Mellange et al. 1992; Y-G. Liu and S.K. Baidoo, unpublished), indicating that the response to enzyme supplementation may vary. The spectrum and concentration of enzymes are therefore important, as their effects are highly specific and influenced by the composition of the diet and the age of animal. More research is required to clarify and establish recommendations on the use of exogenous enzymes in the diets of pigs.

Table 5. Ileal digestibility of essential amino acids in pigs fed hull-less barley supplemented with various fungal-enzyme preparations.

	Control digestibility (%)	+ Hemicellulase		+ Cellulase		+ Cocktail	
		Digestibility (%)	Response (%)	Digestibility (%)	Response (%)	Digestibility (%)	Response (%)
Arginine	59.1	66.2	7.1	70.8	11.7	69.6	10.5
Histidine	60.2	66.7	6.5	70.1	9.9	69.1	8.9
Isoleucine	57.6	66.6	9.0	74.6	17.0	64.6	7.0
Leucine	62.0	67.8	5.8	72.9	10.9	70.8	8.8
Lysine	49.9	59.6	9.7	65.0	15.1	64.5	14.6
Methionine	61.8	66.4	4.6	71.1	9.3	71.5	9.7
Phenylalanine	66.1	69.5	3.4	74.0	7.9	74.1	8.0
Threonine	50.0	58.7	8.7	64.7	14.7	61.5	11.5
Valine	60.0	65.1	5.1	71.2	11.2	69.3	9.3
Mean	59.3	64.9	6.6	69.9	12.0	69.0	9.7

Source: Y-G. Liu and S.K. Baidoo (unpublished).
Note: Diet formulation: hull-less barley, 97.5%; vitamin and mineral premix, 2.5%. Each value is the mean of five measurements.

Growth performance

Table 4 summarizes the results of several studies on the performance responses of pigs to enzyme supplements. Most of the studies recorded daily gain and feed-conversion rates. Some also included the incidence of diarrhea. Reported improvements were 5–45% for daily gain and 3–15% for feed-conversion rate. In general, improvement in feed conversion was more frequently reported than improvement in daily gain. Recent data obtained by S.K. Baidoo and Y-G. Liu (unpublished) showed that feed conversion in response to enzyme supplementation was age related, with improvements being 10% for pigs of 8–20 kg; 5.3% for pigs of 20–40 kg; and 3% for pigs of 40–60 kg. These results agree with those in previous reports. Graham et al. (1988) concluded that the reduction in response to β-glucanase supplementation in older pigs was due to the degradation of mixed-linkage β-glucans before entering the ileum in pigs and that this effect was directly related to live weight. A few experiments demonstrated increases in digestibility but not in growth performance (Thacker et al. 1988). About eight studies reported no response. Some of these nil responses may have been influenced by the composition of the diet; for instance, it is less likely that pigs fed a maize–soya diet (Hogberg et al. 1983) will respond to enzyme supplements as well as pigs fed wheat–barley diets do. The other explanation of the conflicting results may be that the enzyme activities did not target the dietary ingredients well in some of experiments, as the different cereals had different NSP components. More specific enzyme preparations may therefore be required to generate maximum responses (Bedford 1995). For instance, wheat and rye, being rich in arabinoxyloses, require an endo-xylanase for partial hydrolysis of the NSPs, whereas barley and oats, being rich in β-glucans, require β-glucanases.

Possible mechanism

The mechanism of enzyme action in poultry appears to be related to reduction of the viscosity of the NSPs in the digesta. However, in pigs it seems to be much more complicated and has not yet been clearly defined. Typically, enzymes may have the following effects:

- Supplementation of the range of endogenous enzymes that are available in the digestive system, resulting in an improved digestive capacity;

- Disruption of cell-wall structure, leading to increased nutrient availability and changes in the physical properties of NSPs, such as water-binding capacity and viscosity;

- Shift of nutrient availability and NSP digestion to more efficient digestion sites;

- Changes in the composition and content of bacteria in the small and large intestines; and

- Improved efficacy of the animal's own enzymes, which results in reduced maintenance requirements.

Discussion

The inconsistent responses in growth performance described in the previous section indicate a complex interaction between enzymes and substrate, which is further complicated by the age and dietary background of the animal and by the experimental procedure. In poultry, the most commonly accepted mechanism of enzyme action is the reduction of digesta viscosity, which thereby facilitates the interaction between substrate and digestive enzymes. However, the structure and characteristics of the pig's digestive tract differ from those of poultry in many respects. Microflora in the lower part of the small intestine may play an important role in rendering NSPs available to pigs, particularly finishing pigs. The predominant bacterial genus, *Lactobacilli*, can degrade mixed-linkage β-glucans — about 75% of these β-glucans are digested in the ileum. Nevertheless, even in growing–finishing pigs the digestibility of cell-wall components from various fibrous feed ingredients is low. The relative economic benefit of enzymes for piglets, however, is minor, as only 4% of the total feed used is consumed during the 21-d postweaning period, whereas the feed consumed by grower–finisher pigs represents 62–68% of the total feed used. In commercial pig production, even small improvements in feed conversion can result in considerably increased profits. For this reason, enzyme technology should be directed not only to the diets of young pigs but also to the areas in which the greatest overall improvements can be obtained, such as in the digestion of fibrous feeds.

One of the remaining problems with using exogenous enzymes to digest cell-wall components is that they do not target specific substrates. Chesson (1987, p. 71) concluded that the successful use of enzymes was reported mostly in studies in which the problem was "relatively simple and well-defined." To go beyond this will require enzyme formulations designed to target specific substrates. The challenge will be to degrade polysaccharides to a substantial extent. Most dietary-fibre polysaccharides are cell-wall components closely associated with other polysaccharides or noncarbohydrates, such as protein and lignin (Annison 1991). Currently, it is difficult to design an effective enzyme mixture that digests cell walls, as their

makeup is largely unclear and may be variable in different feedstuffs. The structural makeup and physicochemical implications of cell-wall polysaccharides may be determined by two factors: the pattern in which the polysaccharides and other components are arranged; and the bonding between molecules of cell-wall components. Christensen (1989) proposed a general model of the primary plant-cell wall, showing it to be composed of cellulose fibres to which strands of hemicelluloses are attached. Degradation of these complex and insoluble compounds probably requires multiple enzymes (Chesson 1987; Marquardt, this volume). Commercial-enzyme manufacturers have been responsible for the development and production of multienzyme systems. Many of these enzyme preparations can effectively degrade fibre polysaccharides in pig diets and therefore significantly improve the energy availability. However, further developments will be required to produce cost-effective and appropriately targeted enzyme preparations.

Another concern is the effects that pelleting or other types of processing and the conditions in the digestive tract have on the stability of enzyme preparations. Although formulators will take measures to protect enzymes — such as selecting suitable microbial strains, improving production procedures, and providing a substrate carrier to which the enzymes can bind and become immobilized — aggressive processing substantially jeopardizes enzyme activity. For instance, the recovery of β-glucanase decreased from 56% to 31% after the conditioning time was changed from 15 s to 15 min at 85°C and decreased from 16% to 11% at 95°C (Inborr and Bedford 1993). Furthermore, methods to monitor enzyme activity are problematic — there are as many methods as there are enzyme manufacturers, and measurements of enzyme activity in feeds are usually inaccurate and subject to high variation as a result of low enzyme concentrations and relatively poor desorption recovery. Therefore, it is important to develop a reliable and consistently used method for both quality control and rating of the products offered to feedmills and farms.

Conclusion

Feed-enzyme technology has developed considerably in recent years. At present, multienzyme preparations containing β-glucanase for the barley diets of young pigs and phytase to control pollution play important roles. Although there has been this selective use of enzymes in pigs' diets, more complex uses have yet to be elucidated. Also, the efficacy of enzyme treatment in pig husbandry appears uncertain, as well as its cost relative to benefits. More detailed research is needed in the key area of the utilization of fibre polysaccharides to improve energy availability and hence feed efficiency in finisher pigs. Natural products, such as feed

enzymes, may also prove to be effective replacements for antibiotics and growth promoters, which have an adverse impact on human health and the environment.

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Recommendations for future research on the use of enzymes in animal feeds

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Enzymes as additives to animal feedstuffs have had a great impact on the livestock industry. Not only have they improved the utilization of diets containing cereals such as barley, wheat, rye, and oats, especially for poultry, but also they have improved the quality of the environment by reducing the output of excreta and pollutants, such as phosphate and nitrogen, including ammonia. Enzyme supplementation of cereal-based diets has been instrumental in producing more uniform performance values in poultry and tends to be more efficacious with wheat and barley with low apparent metabolizable energy (AME) values than with cereals with higher AME values. Cereals with low AME values often have a high content of growth-inhibiting, viscous, water-soluble, nonstarch polysaccharides (WSNSPs); as a result, the response to enzyme treatment is greater than that obtained with cereals with a lower concentration of WSNSPs. The net effect of adding enzymes to cereal-based diets is, therefore, increased AME values and more uniform performance values. Enzymes have also been shown to decrease the size of the gastrointestinal (GI) tract, which, in addition to increasing the partitioning of nutrients into edible tissue, alters microbial fermentation, which may affect the availability of nutrients. This may in turn affect the health status of the animal.

Most of the research on enzymes as feed additives has involved poultry. Nevertheless, studies with pigs, especially young pigs, have also demonstrated a positive response to enzyme supplements. The recent appearance on the market of recombinant enzymes, especially phytase, should further accelerate the adoption of enzymes by the feed industry. For some recent reviews on the use of enzymes in the feed industry, see Annison and Choct (1991), Bedford (1995), Jeroch et al. (1995), and Marquardt (this volume).

Although enzymes have proven to be highly beneficial, the use of enzymes is still in its infancy. Many problems need to be solved before their full potential is reached. This paper recommends areas of further research.

Areas requiring additional research

Improved enzyme assays

Currently, no standard procedure exists for assessing the quality of commercial enzyme products, nor has a satisfactory assay been developed for monitoring amounts of enzymes in diets. Part of the problem is that many different assays are used to monitor enzyme activity, and assay conditions vary considerably. Also, enzymes, once added to a feedstuff, tend to show low concentrations, with some being bound tightly to the feedstuff (Bedford 1993).

Some of the commonly used enzyme assays include measurements of the reducing sugars liberated by the enzymes; the use of coloured substrates; immunological methods, such as the enzyme-linked immunosorbent assay (ELISA); and assays based on the ability of an enzyme to reduce the viscosity of WSNSPs (Cowan and Rasmussen 1993; Headon and Walsh 1993). Cowan and Rasmussen (1993) reported that among the different methods, the release of dye from the substrate is one of the easiest and most sensitive methods but it is not sensitive enough to readily detect enzymes in feeds. The ELISA procedure can detect enzymes in feeds, but antisera are not available for all of the different enzymes, and ELISA sometimes shows a weak reaction to inactivated enzymes. Measurements of the reducing sugars liberated by the enzymes are inaccurate because of the high background level of reduced sugars in feed. A suitable viscosity assay for feed enzymes has not been reported. Cowan and Rasmussen concluded that the only practical way to assay for enzymes in feedstuffs was to extract the enzyme from the feed and then use the coloured-dye method. This assay, however, must be modified to include an extended incubation time to allow for the low levels of enzyme activity that are usual in feeds.

Although none of the above methods have been shown to be useful for assaying enzymes in feeds, they can all be used to assay enzymes in commercial products before addition to diets. It is not possible, however, to evaluate different products on the basis of stated enzyme activities, as each of the above assays will yield different activity levels for the same product. Also, with any given assay method, activity values may be vastly different from laboratory to laboratory, depending on purity of the substrate, assay conditions, and technical differences. Clearly, what is needed is a standardized enzyme assay that is simple, accurate, and reproducible from laboratory to laboratory. There should also be an associa-

tion between activity values obtained for an enzyme in a feedstuff and its *in vivo* effects.

Government regulatory bodies, enzyme manufacturers, the feed industry, and professional societies should therefore make a concerted effort to develop standardized assays for feed enzymes. This is important, as standardized assays would

- Allow the manufacturers and purchasers of enzymes to compare enzymes based on their activity values;
- Enable livestock producers to determine the amount of active-enzyme product that was added to a diet or the amount that survived the rigours of the environment, including heat treatment during processing;
- Provide a means of assessing how well the enzymes survive in different sections of the GI tract, especially in the section where they are most efficacious [the benefits of this are discussed in the next section]; and
- Bear some relation to the physiological effects the enzymes are supposed to have (for example, it may be possible to run an assay at pH 8 and 60°C, but the results would be irrelevant to the feed industry).

Site of action of enzymes

Fundamental information is lacking on where in the GI tract enzymes produce most of their beneficial effects. It is not known, for example, whether the main site of action of enzymes in chickens is in the crop, proventriculus, gizzard, duodenum, ileum, rectum, or all or part of the GI tract. Preliminary unpublished data (M.R. Bedford, 1996) suggest that in some cases viscosity may be reduced by as much as 60–70% during the conditioning of a wheat-based diet at 85°C. Such information would be most beneficial, as it would assist in the selection of enzymes appropriate for the target substrate and the conditions at the site where they are most efficacious. The type of enzymes selected for use in poultry, for example, could be very different if the major site of action turned out to be the crop rather than the lower sections of the GI tract — the enzymes' ability to resist proteolysis and the optimal pH for their action would be considerations. Also, the optimal properties for specific feed enzymes may vary depending on the species of animal. Enzymes effective in chickens may not be so effective in pigs because of the enzymes' susceptibility to inactivation by a low pH and by proteolytic enzymes.

Production of new forms of enzymes

The current-generation enzymes are highly beneficial, but their usefulness will undoubtedly increase when new forms of the same enzymes are made available. Enzymes from sources other than those currently used, such as those produced by microorganisms in the rumen of cattle or from thermophilic organisms, will have the following properties:

- High activities (substrate turnover rates per unit of protein) under the conditions at the site where they act;
- High levels of resistance to inactivation by heat treatment, low pH, and proteolytic enzymes;
- Low production costs; and
- Long shelf live under ambient storage conditions.

These new enzymes will probably be made available as a result of the production of recombinant enzymes. This involves the cloning and isolation of specific complementary DNA (cDNA), encoding for the specific enzyme of interest, and the transfer of the cDNA to microbial strains producible in large amounts at a low cost in large-scale fermenters (Ward and Conneely 1993). The use of recombinant DNA technology, coupled with site-directed mutagenesis, will allow the production of enzymes tailored to the specific requirements of the target animals. This powerful technology was developed by M. Smith and co-workers, who won the Nobel Prize in 1993 for their seminal work on mutagenesis at a specific position in the DNA sequence (Hutchison et al. 1978).

A new area of research concerns the generation of a second type of tailor-made enzymes. This involves the production of catalytic antibodies from immunoexpression libraries, using a microorganism such as *Escherichia coli* as the expression system. This technology will lead to the production, in fermenters, of antibodies with enzyme-like properties designed to meet the requirements of specific feedstuffs and classes of livestock and poultry (Lerner et al. 1992; Mayforth 1993). Modifications of these procedures will undoubtedly develop. Alternatively, antibodies could be linked to specific proteases to concentrate the enzyme on its target substrate.

Alternative sources of enzymes

Enzymes may be not only directly produced from fungi that have been improved using traditional methods but also expressed in microorganisms, such as bacteria, and in plants, such as in canola seed. An abundant supply of enzymes in canola seed would dramatically reduce the cost to the livestock producer. Production of phytase in canola or xylanase in rye would also result in a higher value feedstuff.

An alternative strategy for the hydrolysis of antinutritive compounds in animal feeds is to construct transgenic monogastric animals able to digest cellulose, β-glucans, xylans, or phytic acid. Forsberg et al. (1993) concluded that it should be feasible to use bacterial DNA constructs to express and secrete glycanases in rat pancreatic acinar cell lines. The major challenge will be to obtain sufficiently high levels of the expression of glucanase gene(s) and other genes in pancreatic cells to effect adequate hydrolysis of the glucans, xylans, etc., in the intestine. Of particular interest would be introducing a phytase gene into domestic animals.

Role of synergistic interaction among enzymes

Nearly all the research done on the response of poultry to enzymes (with the exception of phytase) has used crude fungal extracts with high levels of the desired activities, such as of β-glucanase or xylanase, but also with considerable amounts of other enzymes, including proteases. Research should be carried out using different combinations of pure enzymes (that is, devoid of synergistic enzyme activities) to determine whether the principal enzymes have a synergistic, antagonistic, or additive effect. For example, the ability of enzymes to reduce the viscosity of the water-soluble arabinoxylans in wheat or rye may depend on the amount not only of endo-xylanase but also of arabinofuranosidase, and possibly β-glucanase, acetylxylan esterase, and feruloyl esterase, in the preparation (Forsberg et al. 1993). Preparations high in protease activity might also have a negative effect, as they would enhance protein digestion, including the digestion of the added enzymes. The future availability of cloned enzymes devoid of other enzyme activities will enable researchers to determine the benefits of using various cocktails of enzymes to improve the nutritive values of cereals. Such studies will become more important as attention shifts from the use of crude fungal enzymes to the use of enzymes produced by recombinant DNA technology.

Nature of the interaction of enzymes with dietary constituents

Recent studies indicated that a saturated fat (tallow), compared with an unsaturated fat (soybean oil), in diets with high levels of WSNSPs can dramatically depress

chick performance. Studies by Dänicke et al. (1995), for example, demonstrated that although enzyme supplementation of a soybean diet enhanced growth and fat digestibility, its effects in this diet were completely overshadowed by those obtained with a tallow-based diet. Similar results were reported by Schutte et al. (1995), who observed that responses to enzymes added to diets were affected not only by the viscosity of the WSNSPs but also by other dietary ingredients, such as tallow. Further research should be directed to establishing the nature of these interactions, as this can be expected to provide fundamental new information on the mode of action of enzymes and on the optimization of diets under different dietary conditions, which may in turn be of considerable economic importance.

Modelling studies with enzymes

Zhang et al. (1996) recently showed that they could predict the response of poultry to a particular feed enzyme by using a simple linear model. The model predicts that the response to enzyme supplementation is a function of enzyme concentration when this is converted to a logarithmic value and that a doubling or halving of the response to enzyme treatment can be achieved only by varying the amount of enzyme by a factor of 10, not 2 as may be expected. Zhang et al. also showed that they could simultaneously predict the response to enzyme supplementation of diets containing any given amount of enzyme in any proportion of two cereals. Their model can be readily adapted to least-cost analysis, provided accurate input data are available, and could therefore provide a basis for estimating economic return per unit of enzyme added to a diet. However, further research is required to verify the model, to further simplify its use, and to obtain baseline values. This model may provide a sound basis for predicting responses to any given amount of enzyme for any given feedstuff.

Studies with other animals

Although studies with chickens have clearly established the benefits of enzymes as feed additives, only a limited number of studies have been carried out with other species of poultry, such as turkeys, ducks, geese, and ostriches. Also, very few studies have been carried out using fish, eels, alligators, turtles, other exotic animals, pets, such as dogs and cats, and fur-bearing animals that have simple stomachs. Enzymes may be particularly beneficial in the diets of animals that tend to be carnivorous, as they also tend to have GI tracts with smaller large intestines and therefore do not house a large population of anaerobic microorganisms capable of hydrolyzing complex carbohydrates. Further studies also need to be carried

out using pigs of different ages, particularly with diets having fat from different sources.

Finally, the use of recombinant rumen microorganisms containing over-expressed and bioengineered cellulases and hemicellulases as inoculum for ruminants may increase the efficiency of forage utilization in ruminants. Clearly, enzymes may have many possible uses in other species of animals. As well, there are probably many other enzymes that could be effectively used in the livestock industry.

Target substrates in cereals

Many researchers have hypothesized that the principal mode of action of most enzymes is the destruction of gel-form polysaccharides leached from cereal cell walls in amounts sufficient to depress performance (Annison and Choct 1991; Bedford 1993; Chesson 1993). An alternative explanation was proposed by Hesselman and Åman (1986). They proposed that the β-glucans and arabinoxylans that form the endosperm wall of cereals restrict the access of enzymes to nutrients. They postulated that the disruption of intact walls and the release of entrapped nutrients are the major factors in the improvement of the nutritive value of diets with exogenous enzymes. Chesson (1993) postulated that a single enzyme should be effective if the beneficial effects of enzymes are attributable solely to reduced viscosity. The reason is that viscosity is partially a function of chain length and so it is only necessary to break the chain in a few sites to substantially reduce or destroy its gel-forming capacity. However, if the beneficial effects of added enzymes are due to disruption of intact cell walls and release of entrapped nutrients, rather than reduced viscosity, then many more enzymes may be required. Some of the required enzymes have been discussed above and by Marquardt (this volume). Research should be conducted to establish which of the factors is most important, as they each have important implications for the cost-effectiveness of enzyme use. Experiments involving the use of different combinations of purified enzymes may provide information on whether the viscosity of the diet or the entrapment of nutrients within the cell walls is responsible for the antinutritive effects of NSPs.

Release of monosaccharides from hemicelluloses

As indicated in various papers in this symposium volume, most of the research on the use of enzymes in animal feeds has been directed to finding ways to reduce the viscosity of β-glucans or arabinoxylans in cereals and to release entrapped starch from the hemicellulose matrix. Partial hydrolysis of the hemicelluloses will reduce the viscosity of the different carbohydrates and therefore produce the

desired effects. Future research, however, should be focused on the complete digestion of hemicelluloses to ensure that individual monosaccharides are hydrolyzed. Glucose from cellulose and galactose from the α-galactosides can be readily utilized by most animals, whereas the pentoses cannot be readily utilized by all classes of livestock, especially poultry (Schutte et al. 1991, 1992). Further research on the utilization of pentose, especially by poultry, should also be carried out. The development of procedures for the complete hydrolysis of the hemicellulose fraction of cereals and straw and their utilization would be of great economic importance, as it could provide a basis for using products that are present in vast quantities but cannot be utilized to any appreciable extent by monogastric animals.

Physiological and endocrine-induced changes

Few studies have been carried out to determine the effects of the viscous WSNSPs on the output of pancreatic and other intestinal secretions, including enzymes and bile salts; on the immune response; on hormone levels, including levels of growth hormones, thyroid hormones, and insulin; and on glucose tolerance; or to determine the ability of exogenous enzymes to counteract these effects. Including WSNSPs in diets, with and without enzymes, will allow further interpretation of the effects of viscous nondigestible polysaccharides on physiological processes in animals. This not only will have practical benefits but also may lead to the extrapolation of the results to humans, as there is great interest in the ability of fibrous foods to alter nutrient uptake. Han (this volume) initiated a comprehensive study of this and demonstrated the effects of WSNSPs on many of these parameters. Extensive studies will be needed to completely characterize these effects.

Conclusions

In the past several years, the use of enzymes as supplements in feeds has expanded dramatically. The research that led to this was mostly carried out at universities and research institutions and funded in large part by the industry, in cooperation with government agencies. Although dramatic progress has been made in the past decade on the use of enzymes in the diets of poultry and livestock, additional research will be needed in many areas to exploit the full potential of this very powerful and beneficial technology. Areas of research and development should include the following:

- Development of more sensitive and accurate assays;
- Precise identification of the catalytic properties of enzymes most suited to different classes of livestock and poultry;

- A better understanding of the effects enzymes have on the physiological and endocrine responses of animals fed cereal-based diets;

- A better understanding of the ways enzymes produce their beneficial effects and of the nature of the interactions of enzymes with other dietary components; and

- Development of simple models to predict responses to enzyme treatment.

Many new enzyme products are expected to be developed, in many instances through recombinant DNA technology. They are expected to have superior stability and catalytic properties and to be available at a relatively low cost. They are also expected to find wide application in the diets of many different classes of poultry, livestock, and other types of animals.

Enzymes not only improve animal performance but also have many other beneficial effects, including reduced pollution of the environment. Enzymes as feed additives have had a very great impact on the livestock industry and will continue to provide an ever-increasing range of benefits. There are also many challenges in this rapidly expanding field.

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ochratoxin A; the ability of enzymes to neutralize the antinutritive factors in Canadian feedstuffs; and the development of diagnostic assays to detect several mycotoxins. Dr Marquardt has published more than 200 peer-reviewed papers in national and international journals, written more 18 review papers or chapters in books, made numerous invited presentations at national and international meetings, been awarded five patents, trained many graduate students, and had numerous visiting scientists carry out research in his laboratory. In addition, he has participated in several cooperative programs with researchers from other universities, including this IDRC-sponsored joint research project with Nanjing Agricultural University. Dr Marquardt has been an associate editor and editor of the *Canadian Journal of Animal Science*, served as a member on two grant-selection committees for the Natural Sciences and Engineering Research Council of Canada, and been a member of the Council of Animal Nutrition, US National Academy of Sciences. Dr Marquardt received the Canadian Society of Animal Science's Shur-Gain Award for Excellence in Research in Nutrition and Meat Sciences, the *Sigma Xi* (Manitoba Chapter) Senior Scientist Award, and the University of Manitoba Students' Association Award for Excellence in Graduate Teaching.

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