FROM HYPOTHESIS TO PRACTICE:

USE OF A LOG-LINEAR MODEL TO PREDICT AND EVALUATE THE RESPONSE OF NON STARCH POLYSACCHARIDE ENZYMES IN POULTRY FEEDS

A Thesis

Submitted to the Faculty of Graduate Studies of University of Manitoba

in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

BY

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FACULTY OF GRADUATE STUDIES

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree of DOCTOR OF PHILOSOPHY

谨献

外祖父: 毛家凤
外祖母: 王云秋

爱与哺育，使我沐浴阳光。

父亲: 张宏德
母亲: 毛南针

爱与期望，促我奋发图强。

妻: 朱建辉

爱与支持，助我实现理想。

DEDICATION

To my maternal grandparents, Mr. Jiafeng Mao and Ms. Yunqiu Wang.

Your love and guidance provided me with the foundation

to receive the sunshine throughout my life.

To my parents, Mr. Hongde Zhang and Ms. Nanzhen Mao.

Your love and expectations provided me with the power

to achieve the higher goals in my life.

To my wife, Ms. Jianhui Zhu.

Your love and understanding provided me with the freedom

to realize the dreams of my life.

FOREWORD

"Some of the seed fell among thorn bushes, which grew up and chocked the plants. But some seeds fell in good soil, and plants produced corn; some produces a hundred grains, ..."

-The Parable of the Sower (Mark 4. 1-9; Luke 8.4-8)

The log-linear model is like the seed. It originally fell in the research field of feed enzyme evaluations in the Department of Animal Science, University of Manitoba in 1993 when I was a visiting scholar. Through intensive and careful cultivation over many years, it has finally disclosed some of its potential value in the feed enzyme area. As a result, the thesis, entitled " From Hypothesis to Practice: Use of a Log-Linear Model to Predict and Evaluate the Response of Non Starch Polysaccharide Enzymes in Poultry Feeds," was produced.

The major part of the thesis contains four manuscripts. They are 1) A Simple Model for Predicting the Response of Chicks to Dietary Enzyme Supplementation, 2) Prediction of Effect of Enzymes on Chick Performance When Added to Cereal-Based Diets: Use of a Modified Log-Linear Model, 3) Predicting and Evaluating the Profitable Effect of Feed Enzymes: Use of a Log-Linear Model in Poultry, and 4) Evaluating the Efficacy of Enzyme Preparations and Predicting the Performance of Leghorn Chicks Fed Rye-Based Diets with a Dietary Viscosity Assay. Manuscript 1 mainly demonstrates the rational of why the log-linear model can be used to predict the response of chickens to a feed enzyme and outlines the accuracy of the model. Manuscript 2 mainly focuses on the relationship between the

efficacy of a feed enzyme and the slope of the model when used under different situations. Manuscript 3 is mainly concerned with the prediction and evaluation of profitability rather than productivity. Finally, an in vitro dietary viscosity enzyme assay was developed so as to explore the potential of obtaining values for the log-linear model when used in conjunction with the in vitro system.

This thesis was written in a manuscript format. The first manuscript has been published in the "Journal of Animal Science," the second and the fourth have been published in "Poultry Science," and the third has been accepted in "Poultry Science" for publication. For the purpose of uniformity, all manuscripts were formatted to meet Poultry Science Guidelines for manuscript preparations, however, no other changes were made to the content of each manuscript.

Now, the results of the thesis have also become the seeds. Their values will be recognized, and a hundred grains will be produced in the near future.

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"When you drink the water, think of those who dug the well."

-An Old Saying in China

There are many people who have provided guidance, support and encouragement during the completion of this thesis.

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Ms. Kristina Orzechowski, Mr. Dongsheng Liang, Mr. Thomas Nortey, Mr. Lin Fan, Mr. Steven Cole, and Mr. Suk-Hyeon Cho. Their assistance, suggestions, and kind words were greatly appreciated.

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ABSTRACT

Enzymes have been widely used as a feed additive to improve growth performance of poultry and domestic animal. However, it has not been possible to accurately predict and evaluate the response obtained with a given dose of a specific enzyme preparation. The objective of this research was to determine if a new mathematical approach, a log-linear prediction model equation, could be used to predict and evaluate the response of chicks to a dietary enzyme supplementation. Two dose-response experiments with Leghorn chicks and those from several publications were studied to determine whether a simple general equation could be used to predict the relationship between the amount of a feed enzyme added to a diet and chick performance. An in vitro dietary viscosity assay was developed to determine whether it could be used in conjunction with the model as the predictor or evaluator. The results demonstrated that the model was able to accurately predict (high r^2 values) the response of chicks fed diets containing the different amounts of an enzyme and different proportions of two cereals. The slope of the model was a measure of the efficacy of the feed enzyme. The efficacy, in turn, was able to correctly evaluate the effects of different feed enzymes when added to a diet and to identify the target cereal for an enzyme. In addition, a Multi-purpose Enzyme Analyzer has been developed based on the model. The analyzer was able to determine the optimal amount of an enzyme and a substituted cereal that should be used in a diet for maximal profit, and to determine the amounts and the expected prices of the enzyme and cereal that will yield a given profit. Therefore, the effect of a feed enzyme could be evaluated using maximal profit as a criterion. Thus, the most profitable effect of different

feed enzymes and the cereals that should be used for a given feed enzyme could be determined. Furthermore, a dietary viscosity assay has been developed. The results indicated that there was a linear relationship between the log of dietary viscosity change measured by the assay and the log of amount of enzyme added to a diet ($r^2 = 0.99$, $P < 0.005$). The values from the assay were able to predict the response of chicks to a feed enzyme and also evaluate the efficacy of different feed enzymes, especially for those enzymes that hydrolyzed the viscous compounds in the diet. These studies demonstrated that the response of chicks to a feed enzyme and the efficacy of the enzyme could be predicted and evaluated on the basis of a log-linear model using different criteria (performance and economic return), and different type of studies (in vivo and in vitro).

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LIST OF ABBREVIATIONS

CC	Celluclast
CF	Cereflo
CP	centipoises
CT	Cellulase Tv concentrate
CBD	cellulose binding domain
DM	dry matter
F/G	feed to gain ratio
FZ	Finizym
GI	gastrointestine
h	hour (s)
HMC	high molecular weight carbohydrates
Kcat	turnover number
KD	kilodalton
Km	Michaelis-Menden constant
MEn	Metabolizable energy
min	minute (s)
MRT	mean retention time
NMR	nuclear magnetic resonance
NSP	non-starch polysaccharide
pKa	dissociation constant

SP	SP249
Vmax	maximal velocity
Xyn	xylanase
yr	year (s)
U/g	Unit per gram
WG	weight gain
Wk	week (s)

INTRODUCTION

World feed production for industrialized farming currently tops 575 million tonnes annually with about 57% of the production being for pigs and poultry enterprises (Dunn, 1999, Hofman, 2000). However, only about 6% of manufactured animal feeds contain enzymes. The worldwide feed enzyme business is now estimated to be worth about $100 million, more than 20 times greater than the 1990 value. The potential is even greater (McCoy, 1998). Currently most enzymes that are used in feeds are xylanases for wheat- and rye-based diets and β-glucanases for barley- and oat-based diets. The target of these enzymes are the non-starch polysaccharides (NSPs) that are found in cereals; they include xylanases for xylans and β-glucanases for β-glucans. Phytase is also widely used in some countries (Marquardt, 1997; Ziggers, 1999).

The commercial use of NSP enzymes in the animal feed industry has greatly expanded in the past ten years especially in countries like Canada that utilizes large quantities of cereals such as barley, wheat, triticale, and potential use of rye in poultry and pig diets. As biological catalysts, NSP enzymes are able to neutralize the negative effects produced by certain viscous NSPs in these cereals. These enzymes when added to diets, especially for poultry, have been shown to improve the efficiency of feed utilization, increase the rate of growth, improve the health of the gastrointestinal tract, and reduce environmental pollution due to a decreased output of manure and gases such as ammonia (Choct, 1997; Bedford, 1997b; Marquardt, 1997). Although feed enzymes have been proven to be highly beneficial, the use of enzymes has many problems that must be solved before their full potential is reached. One of the

problems is to develop accurate methods to determine if an enzyme should be added to a diet, secondly to determine if the response to the feed enzyme can be predicted, and thirdly to determine if the selected enzyme is the most cost effective among those available.

World markets are becoming less insular and subject to increasing international competition and opportunities. As a result, the need for international information is greatly increasing. Recently, information on enzyme sources, the activities of major enzymes, optimal pH, thermal stability, recommended levels in diets, and enzyme companies that are directly involved in the worldwide enzyme business has been collected (Hotten, 1991; Ziggers, 1999). This information was able to help those in the feed industry to select the best products that are available. However, it is not possible to rank different enzyme preparations according to their activities as determined in their laboratory since the relationship between the activity reported by a company and degree of improvement in animal performance when added to the diet is low (Marquardt and Bedford, 1997; Ziggers, 1999).

The question may be asked, what standards should we use to ensure that the effects of different enzyme preparations are accurately assessed? Marquardt and Bedford (1997) proposed that enzymes as feed additives should have the following properties:

1) High specific activities (substrate turnover ratios per unit of protein) at the site where they act; and

2) High stability such as the resistance to inactivation by heat treatment, low pH, and proteolytic enzymes during processing, and storage, and when present in the gut.

Enzymes having high specific activities relative to the condition in the gastrointestinal tract should provide a measure of the ability of a feed enzyme to promote an enhanced response

in the animal which, in turn, is a reflection of its efficacy (Lehninger et al., 1993) as feed additives. An enzyme assay, therefore, could be developed in terms of this concept. However, to date, it appears that no standard assay based on this concept has been developed for evaluating the effect of feed enzymes. Nevertheless, the successful development of a method to accurately evaluate the efficacy of feed enzymes will be very important. It would not only greatly assist the feed industry in the selection of enzyme preparations having the highest efficacy, but would also stimulate an improvement of other quality aspects of enzymes. In addition, it would provide both basic and practical information on the use of enzymes which in turn will further enhance the use of enzymes in the feed industry.

This review will mainly discuss the factors that influence the evaluation of the efficacy of NSP enzymes such as xylanases as a feed additive in poultry diets. It will cover the following aspects: developmental history of feed enzymes, mechanism of action of NSP enzymes such as xylanases, the nature of the complex target substrates of xylanases (structure, content, and anti-nutritive properties), the multiplicity of action of xylanases (structure-function relationship), site of action of exogenous enzymes in the gut, and the current methods for evaluating the effects of NSP enzyme preparations and their limitations. Several reviews on different aspects of the above topics have been previously published (Classen and Bedford, 1991; Chesson, 1993; Bedford, 1995; Jeroch et al., 1995; Bedford and Morgen, 1996; Guenter, 1997b; Marquardt, 1997; Marquardt and Bedford, 1997, 2001; and Kulkarni et al., 1999). Finally, the hypothesis and objectives of my research proposal will be briefly discussed.

LITERATURE REVIEW

DEVELOPMENT OF FEED ENZYMES

Enzymes have been widely used in different industries, such as food-processing, brewing, fruit juice-making, baking, leather-working, and washing (detergents), for several decades. For example, in the fruit juice-making or baking industries, enzymes improved yields by as much as one third. An increased understanding of the properties and functions of these enzymes has led to their use in the animal-feed industries, especially for poultry (Guenter, 1997b; Marquardt, 1997).

Enzymes as a means to improve the nutritional value of cereal grains for poultry has been extensively studied since the mid 1950's (Friesen, 1993). The results of early studies on water soaking of barley grain (Fry et al., 1957; Willingham et al., 1959) suggested that the increased nutritive value of water-treated barley for chickens was due to activated β-glucanases of barley. However, the commercial use of feed enzymes in animal nutrition started at the end of the 1980's with the supplementation of β-glucanases to barley-based diets. The feed industry, before commercial β-glucanases were available, had to limit the amounts of barley in broiler diets so as to avoid sticky droppings and poor performance. At first, yeast cultures were mixed with the feed with the intention of having them secrete a cocktail of enzymes when they were present in the gut. Gradually, these cultures have been replaced by well-defined enzymes. Now, commercial enzymes are crude mixtures of activities with known levels of specific enzymes, or cocktails based on mixtures of specific activities (Classen, 1996; Ziggers, 1999).

Most feed enzymes are primarily derived from bacteria (e.g. *Bacillus* sp.) and fungi (e.g. *Aspergillus* and *Trichoderma* sp.). Recently, genes encoding for different enzymes, such as phytases, β-glucanases and xylanses have been expressed in different systems (microorganisms and plants) to enhance the production of specific enzymes and the corresponding nutritional values of the feedstuffs (Marquardt, 1997). The major enzymes, such as xylanases for wheat and rye-based diets, in many commercial enzyme preparations are often labeled as if they have the same function in the hydrolysis of their target substrates. These enzymes, however, are often different in pH optimums, substrate preferences, temperature optimums and thermal stabilities. In addition, the reaction conditions where these exogenous enzymes act in the gut are determined by nature with very limited possibilities for modification (Classen, 1996; van de Mierop and Ghesquiere, 1998). Consequently, the efficacy of most enzyme preparations varies considerably for a specific diet. In addition, the structure and content of the target substrates within and between cereals for enzymes are complex and vary in nature. Therefore, successful evaluation of the efficacy of a feed enzyme relies on the knowledge of the target substrates, mechanism of action, the nature of the enzyme, and the conditions in the intestine under which the reaction occurs.

MECHANISM OF NSP ENZYME ACTION

There is considerable evidence that supplementation of cereal-based diets for poultry with exogenous enzymes can overcome some limitations of these cereals for digestion and absorption of nutrients. The limitations are mainly a result of the physical and chemical structure of NSPs in these cereals, their anti-nutritional activity, and the limited time for

hydrolysis of nutrients in the gastrointestineal tract. Enzymes have been shown to improve the efficiency of animal production, improve the nutritional value of lower quality feedstuffs, reduce the excretion and waste of nutrients, and maintain beneficial microflora in the gut (Choct and Annison, 1992; Jeroch et al., 1995; Choct et al., 1996; Marquardt, 1997).

A question that has to be asked is, what is the mechanism of action of enzymes that enable them to overcome these limitations? Two hypotheses for the action of NSP enzymes have been proposed:

1) they improve the physico-chemical conditions of the digestive contents (e.g., viscosity reduction); and

2) break down cell walls allowing digestive enzymes access to nutrients (release of entrapped nutrients).

The first hypothesis proposes that the predominant NSPs of cereals are arabinoxylans in rye, wheat and triticale and β-glucans in barley and oats. Both NSPs are partially soluble in the gastro-intestinal tract, resulting in an increased viscosity of digesta (Anitoniou and Marquardt, 1982). This in turn reduces the diffusion and interaction of endogenous enzymes with their substrates and increases the resistance to passive diffusion of digested nutrients across the cell wall of the villi lining the small intestine, thereby altering digestive efficiency (Fengler and Marquardt, 1988b; Choct and Annison, 1990). In addition, the increased viscosity in the small intestine has been shown to increase the time required for feed to pass through the digestive tract (Almirall and Esteve-Garcia, 1994). This in turn increases the concentration of microorganisms (Choct and Annison, 1992; Choct et al., 1996). Two negative consequences of the change in the number of microorganisms are deconjugation of bile salts which can

reduce fat digestion, and the production of toxins. The overall consequences of the viscous NSPs are a reduction in growth rate and feed efficiency, a deterioration of barn environmental conditions due to production of wet, sticky excreta, and an increased incidence of dirty eggs (Classen, 1996). The effects of the exogenous NSP enzymes can be mainly ascribed to their ability to reduce the viscosity of digesta in the intestines. Therefore, NSP enzymes with appropriate activities have been used to successfully enhance the nutritional value of oat, barley, rye, triticale, and wheat for poultry. On the other hand, NSPs may work in another way as they may prevent nutrient release through the complex structure of the cell wall of cereals. NSPs, such as arabinoxylans and β-glucans, which are the major components of endosperm and aleurone, are mainly insoluble. They can therefore prevent the release of nutrients from the cell (Petterson and Aman, 1989).

It is well documented, however, that the viscosity effect in cereal-based diets is the major problem rather than that of the impervious nature of the cell wall (Annison, 1991; Choct and Annison, 1992; Bedford and Morgan, 1996). Firstly, if this latter hypothesis was correct, the action of NSP enzymes in a cereal-based diet would be only expected to increase starch digestibility rather than that of other nutrients. The data do not support this expectation (Fengler et al., 1988; Carré et al., 1992). In addition, the results from a study, in which isolated wheat NSP were added back to a sorghum-based diet, indicated that the negative effects of viscosity were equally apparent on starch and protein digestion, and even more apparent on fat digestion (Choct and Annison, 1992). Secondly, pelleting and expansion of the diet have been shown to increase the response of birds fed NSP enzyme supplemented diets. Such treatments not only increase the release of entrapped nutrients by damage or

perforation of the cell wall, but also increases intestinal viscosity in birds fed these diets (Teitge et al., 1991; McCracken et al., 1993). These results indicated that cell wall integrity is not the major problem, but perhaps that dissolution of arabinoxylans is; again this favors a viscosity-based hypothesis (Bedford and Morgan, 1996).

It is important to understand the mechanism of action of NSP enzymes in order to accurately evaluate their efficacy. The two hypotheses are not necessarily independent of one another, but a preponderance of one effect over the other has significant consequences for the evaluation of enzymes. If viscosity reduction is required, then soluble rather than insoluble NSPs should be the target, and perhaps only one enzyme rather than a multi enzyme combination would be required. Viscosity reduction can be effectively achieved with a single enzyme that has a limited ability (only a few cut on a NSP molecule) to hydrolyze the NSPs of concern (Bedford and Classen, 1992). However, if disruption of intact cell walls and release of entrapped nutrients make a greater contribution to the antinutritive properties of NSPs, then many more enzymes would be required in order to provide access to nutrients that may be entrapped in a NSP matrix (Chesson, 1993). Consequently, different mechanisms may result in different methods for evaluating the efficacy of feed enzymes.

COMPLEX TARGET SUBSTRATES OF XYLANASES: STRUCTURE, CONTENT, AND PROPERTIES

Grain Structure and NSPs

The structure of a cereal kernel is shown in Figure 1. It is comprised of the caryopsis and the enclosing hull or husk. The caryopsis consists of the pericarp, aleurone layer, endosperm

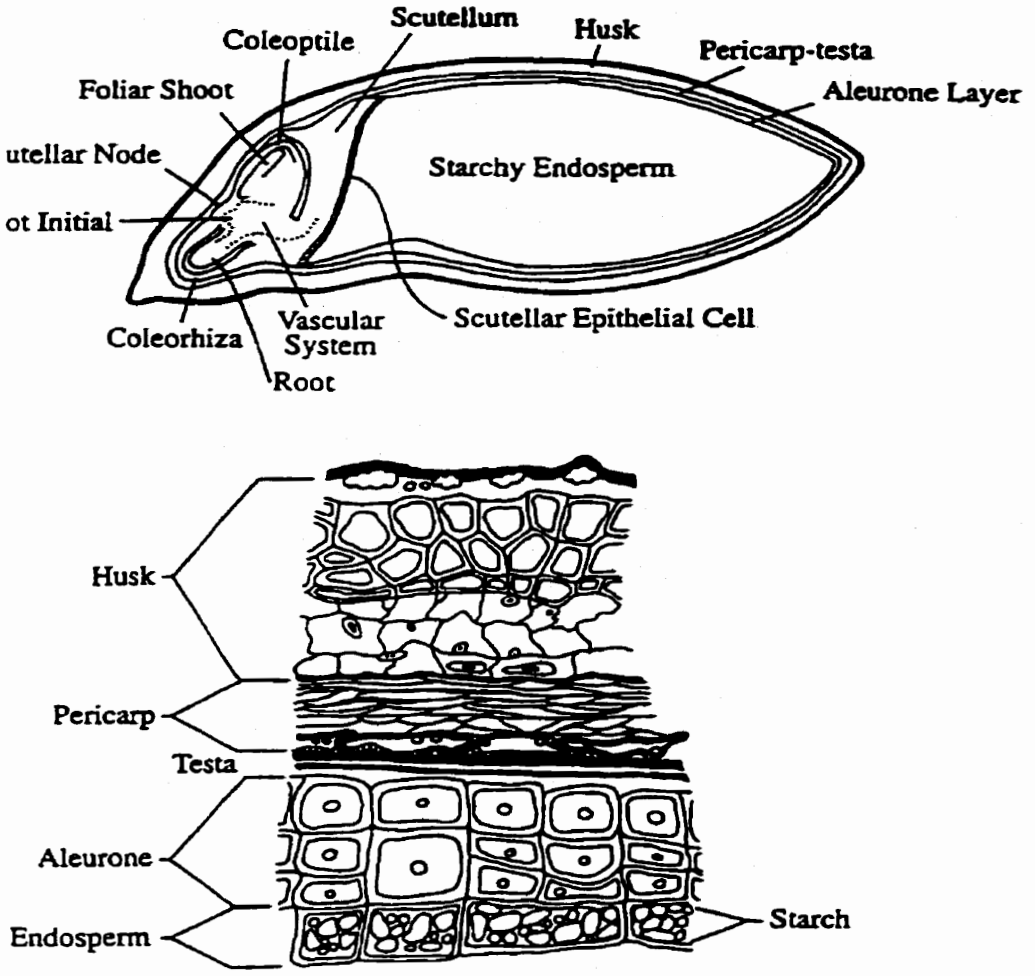


FIGURE 1. Anatomical structure of cereal grains (Source: Kent and Evers, 1994).

and germ or embryo. The most important nutrient in cereal grain is starch, which accounts for approximately 49 to 70 % of grain weight (Chesson, 1993). The whole endosperm is enclosed by a thin layer of cells with very thick cell walls, known as the aleurone layer. It contains enzymes for digestion of the endosperm during germination. The outside of the aleurone layer is the pericarp which acts as a protective cover over the entire grain (Jadhav, et al, 1998). Thus, in order for a non-ruminant to access the starch contents of a grain, it must be able to penetrate the three barriers: the tough or fibrous pericarp, the aleurone layer and the endosperm cell wall.

Cell walls are primarily composed of the NSP carbohydrate fractions with lesser amounts of protein, lignin, and polyphenols (Classen and Bedford, 1991). The carbohydrate fractions consist of cellulose microfibrils embedded in non-cellulose polysaccharides. Cellulose is a small proportion of grain cell walls and is thought to be of little nutritional value for poultry. The major NSP carbohydrate fractions are derived from heteropolymers such as β-glucans and arabinoxylans (pentosans) with both being present in most grains, but their total amount and proportions varies considerably (Henry, 1985; 1987). Arabinoxylans in the cell walls of wheat, rye, and triticale are present at higher concentrations, while the β-glucans are predominant in barley and oats. Protein and phenolic acids are both present in small amounts but may play an important role in cell wall stability.

Content of Arabinoxylans in Cell Walls

Rye and wheat are characterized by endosperm walls in which arabinoxylan is dominant compared to β-glucans in barley and oats (Table 1). Arabinoxylans consist of a backbone of β-1,4-linked xylopyranosyl residues with terminal 1, 2 and 1, 3 arabinofuranosyl substitution.

TABLE 1. The total and water-soluble arabinoxylan and β-glucan content of cereal grains (g/kg) and the contribution of the arabinoxylans and β-glucans in the starchy endosperm walls as a percentage (%) of the dry matter content of the grain[1]

Cereal	Arabinoxylans			β-glucans		
	Grain total	Grain soluble	Endosperm (%)	Grain total	Grain soluble	Endosperm (%)
Rye	84.9	26.0	44	18.9	6.8	71
Wheat	66.3	11.8	35	6.5	5.2	48
Barley	56.9	4.8	22	43.6	28.9	99
Oats	76.5	5.0	12	33.7	21.3	47

[1]Source: Chesson, 1993.

Arabinofuranosyl substitution reduces the ability for hydrogen bonding between carbohydrate chains and consequently results in fractions which are water soluble and highly viscous. A considerable degree of variation in the contents of soluble arabinoxylans or β-glucans has been reported. This occurs not only among the different cereals but also among varieties within a given cereal (Table 2). The content of soluble NSPs in a given cereal varies with method of isolation, variety, soil and climate, agronomic practices, degree of maturity at harvest, storage condition, etc. (Henry, 1986; Saastaminen et al., 1989).

Structure of Arabinoxylans

Arabinoxylans differ between and within grains with respect to the degree of arabinofuranosyl substitution and other characteristics (Table 2). For example, Bengtsson et al. (1992) indicated that there were two type of arabinoxylans in rye grain, arabinoxylan I and II. The xylan backbone chain when substituted with mono-arabinose terminal residues has been classified as arabinoxylan I, whereas the backbone chain when substituted with double-arabinose terminal residues has been classified as arabinoxylan II (Figure 2). In general, arabinoxylans found in the bran or outer aleurone layer have fewer arabinofuranosyl substitutions and are less water soluble than those found in endosperm fractions (McNeil, et al., 1975; Henry, 1987; Hromadkova, et al, 1987). The degree of arabinofuranosyl substitution influences not only the ability of the arabinoxylan to bind to other cell wall constituents but also its susceptibility to hydrolyzing enzymes. Water-soluble arabinoxylan from rye has only 50 % substitution of its xylopyranosyl residues at position 3 while about 2 % of the residues are double substituted at positions 2 and 3 (Bengtsson and Aman, 1990). In contrast, arabinose substitution in wheat tends to occur more frequently at the 2 and 3

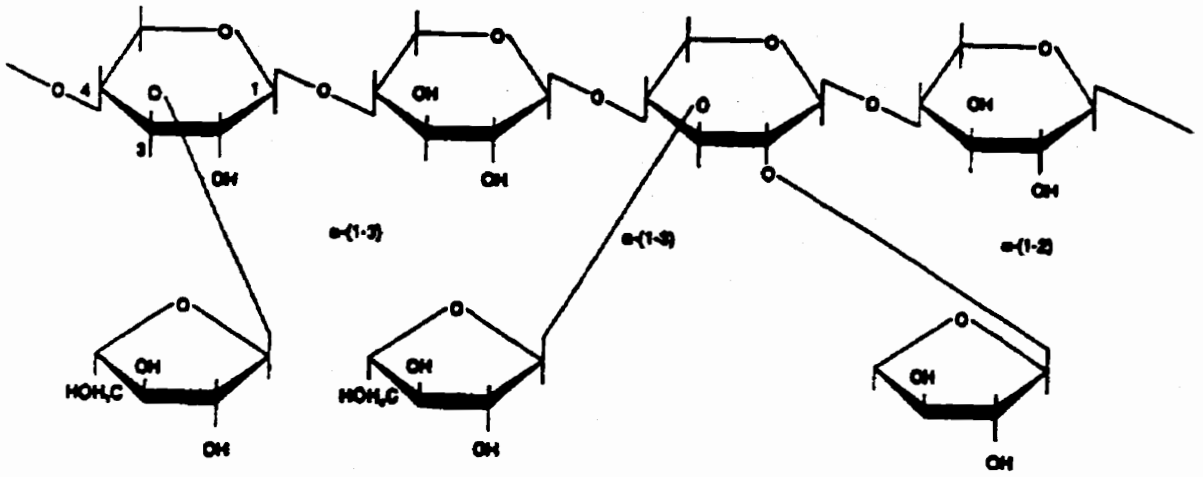
TABLE 2. Variation in content, structure, and molecular weight of non-starch polysaccharides (NSPs) in six European wheat flours[1]

European Wheat Flours	Range
NSP Contents (% Dry Basis)	
Water soluble	0.42~0.69
Total	1.35~2.25
L-arabinose	28.1~31.5
D-xylose	46.6~64.2
D-galactose	0.7~2.3
D-glucose	2.5~9.0
Structure	
L-arabino/ D-xylose	
Water soluble	0.73~1.02
Total	0.59~0.76
Water Soluble NSP [1]	
Di/Mono	0.80~1.81
X_0	63.1~66.1
X_1	13.1~20.5
X_2	16.2~23.7

[1]Source: modified from Cleemput et al., 1993.

[2] Di/Mono = disubstituted/monosubstituted D-xylose, X_0 = unsubstituted D-xylose, X_1 = monosubstituted D-xylose, X_2 = disubstituted D-xylose.

β-(1→4)-D-xylan backbone



α-(1→2),(1→3)-L-arabinosyl side chains

Arabinoxylan I

$$-[X]_m-X-X-[X]_n-X- \qquad m \geq 1,\ n \geq 1$$
$$\qquad\quad\ \ |\ \ |\ \ \ \ |$$
$$\qquad\quad\ \ A\ \ A\ \ \ \ A$$

Arabinoxylan II

$$\begin{array}{c} AA \quad AA \quad AA \\ \vee \quad\ \vee \quad\ \vee \\ -X-X-X-X-X-X-X- \\ \quad\ \wedge \quad\ \wedge \\ \quad AA \quad AA \end{array}$$

FIGURE 2. Structure of arabinoxylan {α-(1→2,3)-L-arabinofuranosyl-}-β-(1→4)-D-xylopyranan (top) and the structural features of arabinoxylans I and II in rye grain (bottom). A, terminal α-L-arabinofuranosyl; X, 4-linked β-D-xylopyranosyl residues (Source: Bentsson et al., 1992; Marquardt, 1997).

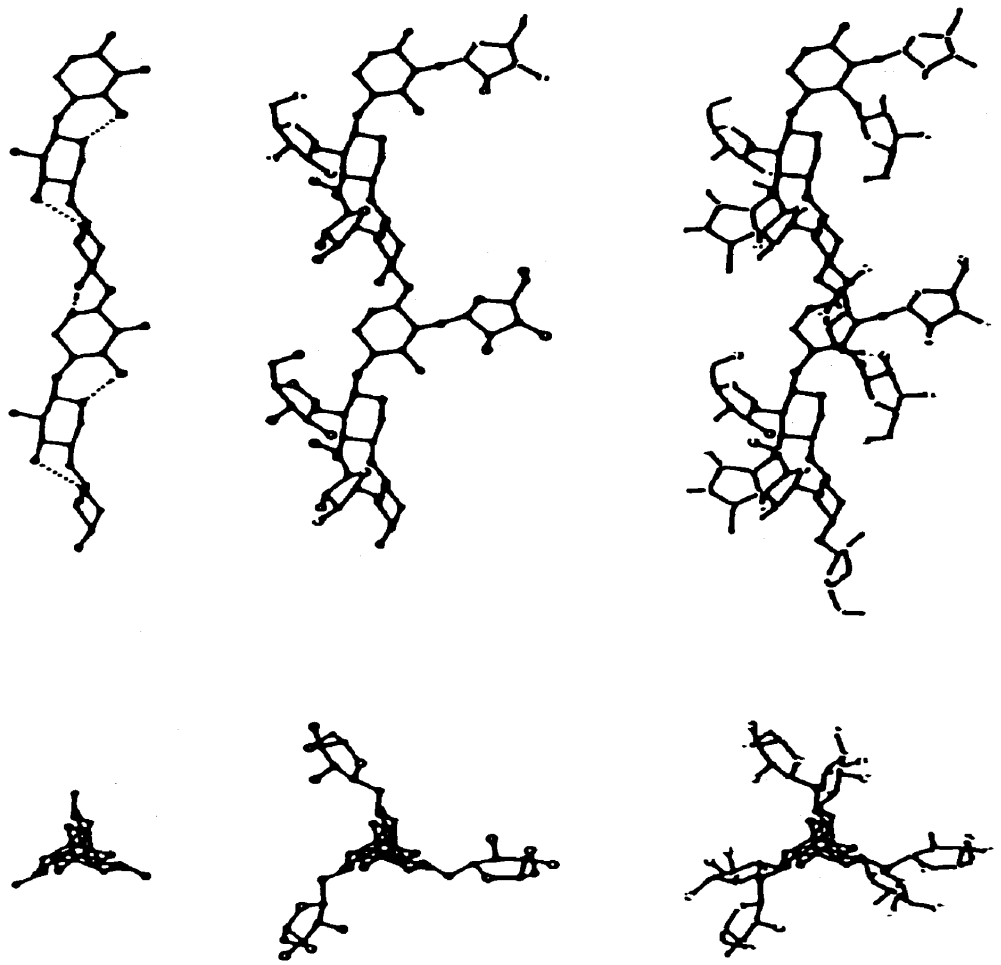
positions compared with rye; the values being 55 and 8 % of xylopyranosyl residues, respectively (Classen and Bedford, 1991).

The three-dimensional structure of xylan has been elegantly described by Atkins (1992). The xylan backbone shows a threefold left-handed conformation when crystallized. The geometry of the glycosidic linkage is not affected by the side chains. The chains are organized in a trigonal lattice with hexagonal morphology. The three-dimension structures of xylan molecules containing none, one or two arabinose substitutions are shown in Figure 3. Knowledge of the structure of xylans has provided an insight into the understanding of the mechanism of reaction between xylanases and xylans.

Xylans are heteropolysaccharides with a homopolymeric backbone chain of β-(1→4) linked β-D-xylose unit. Based on the common arabinose substitutions found on the backbone, the cereal xylans are categorized as arabinoxylans. However, other substitutions such as O-acetyl, α-(1→2)-glucuronic or 4-O-methylglucuronic acid have also been found. In addition, feruloyl groups may form crosslinks between xylan and lignin through substitution (Timell, 1965; Das et al., 1984; Scalbert et al, 1985; Mueller-Harvey et al., 1986). Thus, the side chains determine the solubility, physical conformation and reactivity of xylans with other hemicellulosic components.

Anti-nutritive Properties of Water Soluble Arabinoxylans

Previous studies have indicated that, water-soluble NSPs were mainly responsible for the anti-nutritive properties of wheat, rye, triticales, barley and oats (Annision and Choct 1991; Campbell and Bedford 1992; Bedford 1995; Jeroch et al., 1995). There appears to be a good association between the growth-depressing effects of cereals and their content of viscous



a: xylan backbone without substitution

b: backbone with one arabinose substitution

c: backbone with two arabinose substitution

FIGURE 3. Three-dimensional structure of xylans. Projections perpendicular (top) and parallel (bottom) of xylan backbone. Hydrogen bonds are shown dotted. In each case the backbone is a left-handed threefold helix (Source: Atkins, 1992).

water-soluble NSPs (Marquardt, 1997), and also a high correlation between the amount of soluble arabinoxylans in these cereals and extract viscosity (Boros et al., 1993). Bengtsson et al. (1992) found that the type of arabinoxylans may also affect viscosity. They demonstrated that arabinoxylan II from rye was considerably more viscous than arabinoxylan I. In addition, Bedford and Classen (1992) demonstrated that there is a negative linear correlation between chick performance and the log of viscosity of digesta in the small intestine. Intestinal viscosity was mainly associated with the concentration of soluble high-molecular-weight carbohydrates (HMC) in the gut. The molecular weight of HMC was more than 500 KD. Overall, it has been well documented that the content, structure, and molecular weight of arabinoxylans between or within cereals varies considerably. This in turn greatly influences the mode and extent of enzymatic cleavage.

MULTIPLICITY OF XYLANASES : STRUCTURE AND FUNCTION

The xylanases used in the feed industry come mainly from different species of fungi (eg. *Trichoderma* and *Aspergillus*) and bacteria (eg. *Bacillus*) (Bedford, 1997a; Ziggers, 1999). These microorganisms differ substantially in their requirements for growth. As a result, the xylanases they produce are different in their properties.

Biochemical Properties

Microbial xylanases are single subunit proteins with molecular weights in the range of 8 to 145 KD (Sunna and Antranikian, 1997). The optimal temperature for endoxylanases from bacterial and fungal sources varies between 40 and 60 C. Fungal xylanases are generally less thermostable than bacterial. Xylanases from different organisms are usually stable over a wide

pH range (3-10) and show optimum pH in the range from 4 to 7. The xylanases from fungi such as *A. kawachii* (Ito et al., 1992) and *Penicillium herque* (Funaguma et al., 1991) exhibit optimum pH towards the acidic side (pH 2 to 6). In contrast, the xylanases produced by *B. subtilis* has a pH optimum of above 6, which enables the enzyme to act very efficiently at a neutral or slightly acidic pH. In addition, bacteria are also known to produce two xylanases, high molecular weight having acidic optima and low molecular weight xylanases having basic optima. However, this type of relationship is not observed in fungi, but low molecular weight basic xylanases are common. The amino acid compositions of xylanases reported from various sources are predominantly aspartic acid, glutamic acid, glycine, serine and threonine (MacLeod et al., 1994; Krengel and Dijkstra, 1996; Kulkarni et al., 1999).

The occurrence of glycosylated enzymes is a common phenomenon among many eukaryotic xylanases (Funaguma et al., 1991). The xylanases from prokaryotic sources have also been found to be glycoproteins (Berenger et al., 1985; Marui et al., 1985; Dey et al., 1992). Glycosylation has been implicated in the stabilization of glycanases against extreme environments (Merivuori et al., 1985). The recombinant xylanase expressed in *Escherichia coli* from an alkaliphilic and thermophilic *Bacillus* sp. (Kulkarni et al., 1995) showed lower stability at higher temperature and reduced ability to bind xylan compared to xylanases from the parent strain; this is attributed to deglycosylation.

Substrate Specificity

Knowledge of the mechanism of action of xylan-degrading enzymes has been obtained from studies on substrate specificity, the role of side chain substitutions on their activity, the specificity for bonds that are cleaved and the end products. The xylanases of fungal origin are

well studied. They are mainly of two types; non-debranching enzymes which do not liberate arabinose, and debranching enzymes which liberate arabinose from the side chain substitutions, in addition to cleaving the main chain linkages (Reily, 1981). Many xylanases of fungal origin, such as *A. niger* (Takenishi and Tsujisaka, 1973), were found to release arabinose from arabinoxylan. However, xylanases from another strain of *A. niger* did not release free arabinose from arabinoxylan.

It is commonly observed that substitutions in the highly branched polysaccharides interfere with xylanase activity. However, enzymes having more affinity for main chain linkage, near branch points, were reported from *A. niger*, *T. viride* and other sources (Dekker and Richards, 1976).

Domain Structures of Xylanases

Generally, xylanase is comprised of functional or non-functional domains and linkage regions. The functional domains are further dissected into the catalytic and the substrate-binding domains. It had been assumed that the catalytic domain and the substrate binding domains of the xylanases were spatially separate or distinguishable. However, an analysis of a family 10 xylanase (Xyn A) has revealed that the spatial separation of the protein domains is not necessary (Ferreira et al., 1990). The substrate binding domain was found to be a cellulose binding domain (CBD) with no affinity towards xylan. Truncated derivatives of a xylanase, Xyl A, that lacks the cellulose binding domain were found to be less active against xylan contained in a cellulose-hemicellulose complex as compared with the full length xylanase (Black et al., 1996).

In general xylanases consist of a single catalytic domain. However, two catalytic domains

have also been reported (Black et al., 1996). The amino acid sequence of endoxylanase from *Cryptococcus albidus* has some homology with the catalytic region of egg white lysozyme (Morosoli et al., 1986). The catalytic domain of the xylanase from *C. saccharolyticum* was found to be homologous with the catalytic domain of cellubiohydrolase, bifunctional exocellulase, endocellulase and xylanase from other microorganisms (Boucher et al., 1988; Grapinet et al., 1988; O'Neill et al., 1988; Luthi et al., 1990). At the same time , a striking absence of homology between two xylanases derived from *B. circulans* (Yang et al., 1989) has also been documented.

Structural studies using NMR (nuclear magnetic resonance) have revealed the presence of very few charged amino acids and an unusually large number of conserved aromatic residues in the cellulose binding domain. NMR analysis and chemical modification studies confirmed that the exposed aromatic residues play a direct role in binding to cellulose; their interaction with cellulose appeared to be reversible (Bray et al., 1996). The domain seems to play two possible roles, i.e., they either open the structure of the plant cell wall making it more accessible to enzymic hydrolysis or provide a general mechanism by which a consortium of hydrolases accumulate on the surface of the plant cell wall, resulting in synergistic action between the enzymes. Full length and truncated forms of xylanase D from *C. fimi* were found to be equally effective in hydrolyzing pulp xylans (Rixon et al., 1996). The deletion of the CBD was found to abolish the cellulose binding capacity of the enzyme without affecting the xylan binding properties and xylanase activity. However, the Km value of the truncated xylanase for insoluble xylans was higher than that of the natural xylanase (Black et al., 1995). In addition, CBD of Xyl A from *Thermotoga maritima* exhibits extremely high intrinsic

stability. For example, the apparent denaturing temperature of the xylanase protein exceeds 100 C (Doris et al., 1997).

The Three-dimensional Structure of Xylanases

The three-dimensional structures of many xylanases from both bacterial and fungal species have been determined using x-ray diffraction techniques. The structure of xylanases are similar although they are from different sources and have different molecular masses. The enzyme molecule is of ellipsoidal shape with a well-defined cleft down one side (Fig 4). Generally, it consists of a single domain where the main chain folds to form two mostly antiparallel β-sheets. The two β-sheets are twisted and form a large cleft which is the putative binding sites for at least four xylose residues. The two conserved glutamate residues, for example, Glu^{79} and Glu^{170} of xylanase I from *A. niger*, reach into the cleft from opposite sites (Krengel and Dijkstra, 1996) to form the active site of the xylanase.

In addition to conserved residues in the active site of xylanase, there are a number of other conserved residues. Many of these residues are believed to be structurally important. The catalytic residues of Xyn II from *T. reesei* are Glu^{86} and Glu^{177}. Around Glu^{86}, a clear cluster of conserved residues consists of Glu^{136}, Tyr^{77}, and Tyr^{88}. However, the residues around Glu^{177} are much less conserved (Torronen et al., 1994).

Catalytic Mechanism of Xylanases

It has frequently been suggested that the catalytic mechanism of glycosidase action resembles that of lysozyme (Kelly et al., 1979). The hydrolysis reaction catalyzed by xylanases as well as cellulases proceeds through an acid-base mechanism involving two residues. One of the residues acts as a acid-base catalyst and protonates the oxygen of the glycosidic bond.

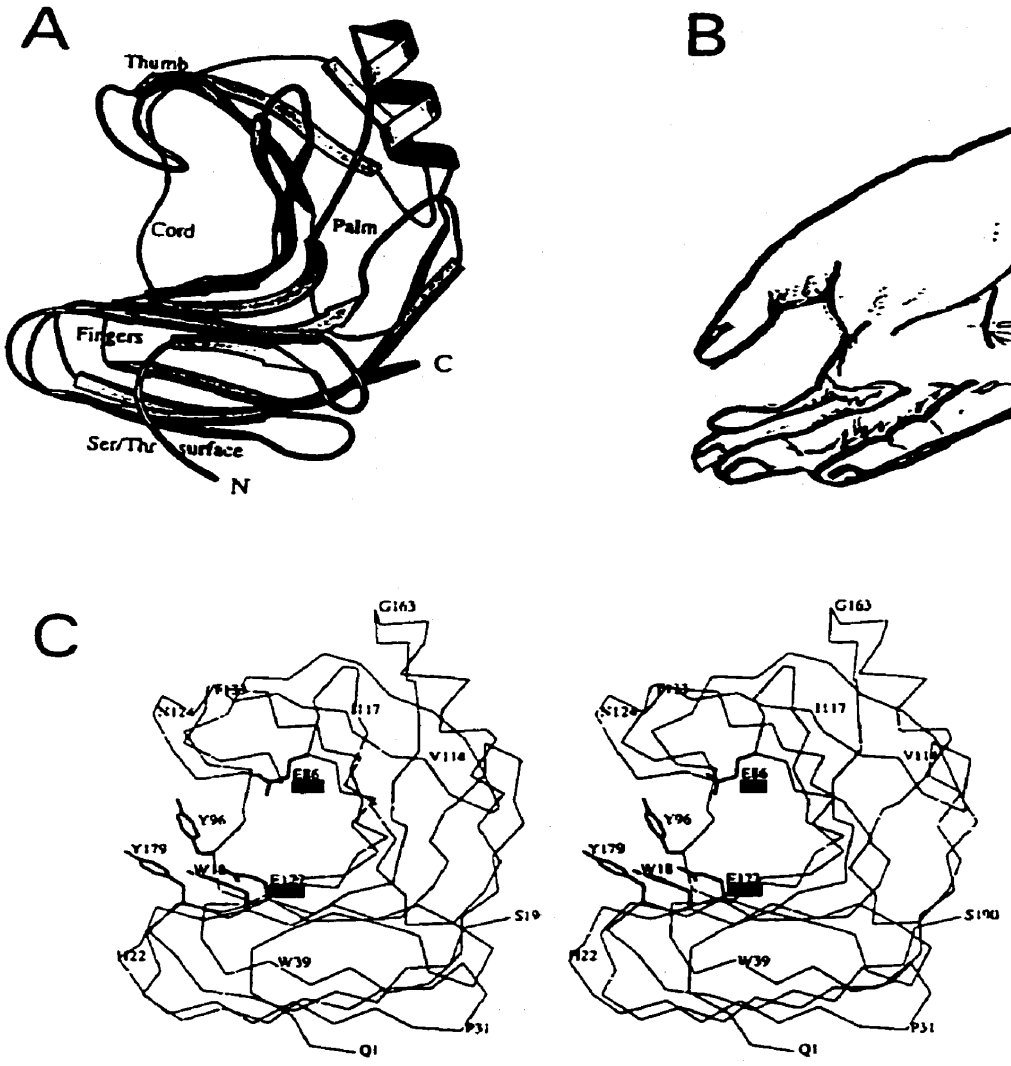


FIGURE 4. (A) Ribbon representation of the xylanase (XYN II) molecule showing an α-helix and β-strands. The structure is reminiscent of the shape of a 'right hand' (B) and this analogy is shown. (C) The Cα skeleton of XYN II in stereo. The putative catalytic residues (Glu86 and Glu177) and aromatic binding residues (Tyr96, Tyr179 and Trp18) are shown (Source: Torronen et al., 1994).

The other residue acts as a nucleophile which interacts with the oxocarbonium intermediate or promotes the formation of an OH^- ion from a water molecule. The reaction involves a two step mechanism in which proton transfer occurs to and from an oxygen atom in the equatorial position at the anomeric center (Figure 5, Lawson et al., 1997). Therefore, the distance between the two catalytic carboxylic acids are essential for the efficacy of xylanases. For the xylanase from *B. circulans*, this critical distance is 5.5 Å. Previous studies identified that Glu^{78} of the xylanase was the nucleophilic residue (Miao, et al., 1994, Lawson, et al., 1996) and the Glu^{172} was the acid/base catalyst. In order to alter the distance between Glu^{78} and Glu^{172}, a strategy of selectively replacing Glu^{172} with its shortened or lengthened analogue was used by Lawson et al. (1997). Glu^{172} was substituted with aspartic acid as its shortened analogue using site-directed mutagenesis. To produce the lengthened Glu^{172} analogue, the combined techniques of site-directed mutagenesis and chemical modification were employed. A cysteine was introduced at the position of Glu^{172} and was selectively carboxymethylated with iodoacetic acid, yielding a carboxylic side chain which was approximately 1.6 Å longer than Glu^{172}. Their results indicated that an increase in the separation (Glu172Asp) resulted in a 400-fold decrease in the Kcat (turnover number) value that represents the catalytic activity of a typical enzyme for xylan hydrolysis. By contrast, a decrease in separation (Glu172Cys) caused only a 25-fold reduction in the rate of xylan hydrolysis (Wakarchuk et al., 1994). The reduction of catalytic activity of the mutants was partly associated with an increase in the pKa value of Glu^{172}, compared with that of the native xylanase. For example, the pKa values are 6.8, 8.0, and 6.7 for Glu 172, Glu172Asp and IAA-Glu172Cys, respectively. In addition, the high correlations of log Kcat /Km with the aglycone-leaving group ability was demonstrated

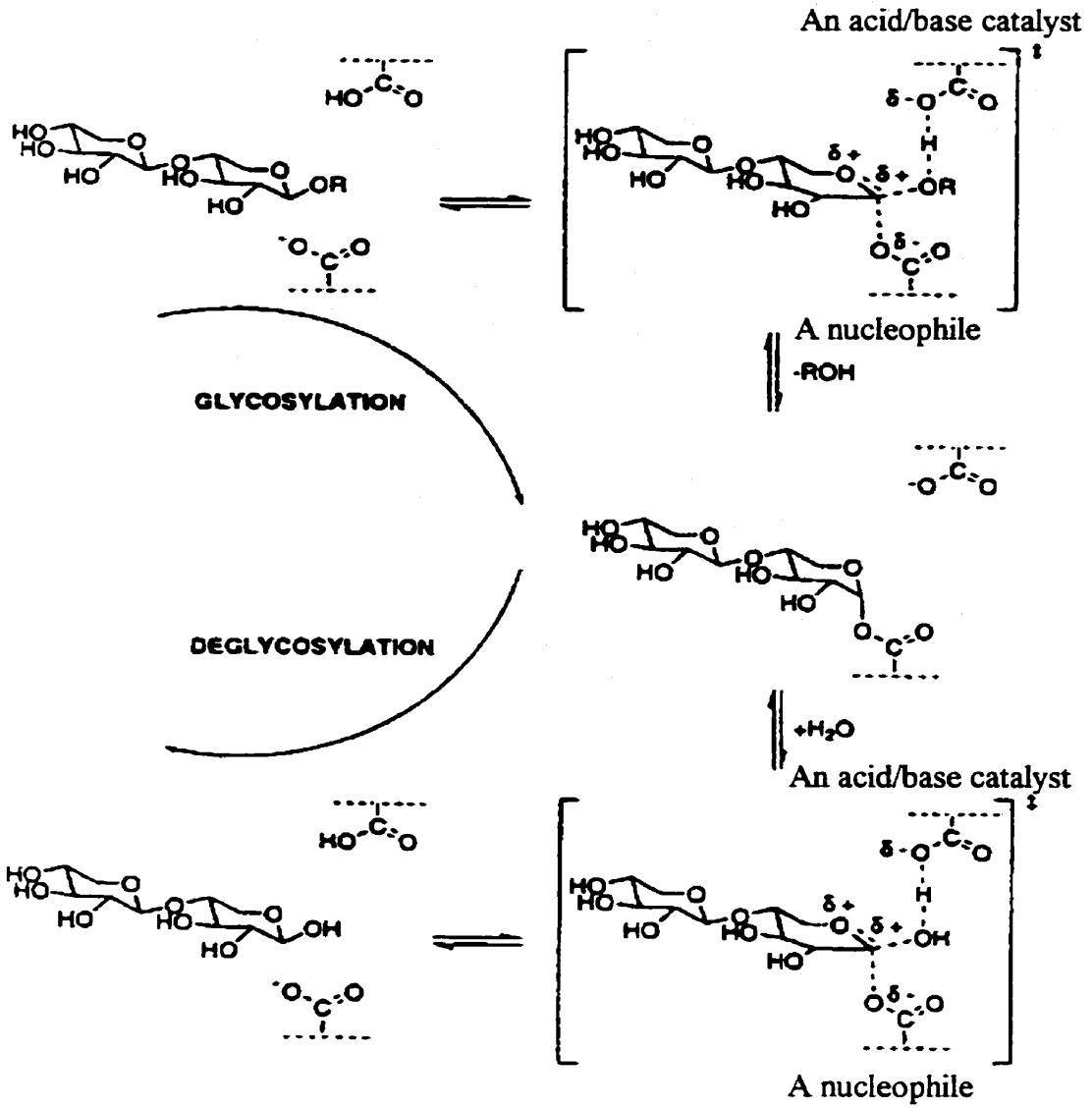


FIGURE 5. Proposed mechanism of action of xylanases. OR represents the aglycon where R corresponds to an aryl group or more xylose residues (Source: Lawson et al., 1997).

for the three enzymes for a range of aryl xylobioside substrates. This indicates that Kcat / Km reflects the first irreversible step, glycosylation, since this step involves sugar aryl bond cleavage. Therefore, the catalytic properties of the whole xylanase may be directly affected by the pKa value of its acid/base catalytic residues. Torronen et al. (1994) demonstrated that Xyn II from *T. ressei* have two conserved catalytic residues, Glu^{78} and Glu^{177}. There is also a clear cluster of conserved residues around Glu^{86}. The situation is different around Glu^{177}, where the surrounding amino acids are much less conserved. The pKa value for 'free' glutamic acid is 4.6 to 5.0. Xyn II has been reported to be most active in a pH range of 5.0 to 5.5 (Tenkanen et al., 1992; Torronen, et al., 1992). In other xylanases, the pH optimum has been reported to vary between 3.5 to 7.0 (Wong and Saddler, 1992). The variations are probably due to the different amino acid residues in the neighborhood of Glu^{177}. A clear structural change of Xyn II was observed especially in the position of the side chain of Glu^{177} when determined at pH 5.0 (optimal pH of Xyn II) and pH 6.0, where enzyme activity is reduced considerably. The distances between two catalytic residues of Xyn II were 10.7 Å at pH 5.0 and 8.1 Å at pH 6.5.

Therefore, the efficacy of different xylanases as feed enzymes varies considerably. Efficacy is determined by the distance between the two amino acid residues at the active site of the enzyme, the pKa value of the residue as an acid/base catalyst, the binding ability of the enzyme to the complex target substrates, and even the stability of the enzyme during the processing or storage periods. It is also dependent on whether the substrate of a xylanase is a highly branched or less substituted structure, or how it interacts with other hemicelluloses or cellulose. Furthermore, the conditions under which xylanase acts greatly influences its

efficacy. Whatever the sources of xylanases, the overall efficacy of the enzyme as a feed additive will greatly be influenced by the reaction conditions in the gut where the enzymes act. Therefore, successful evaluation of the efficacy of an enzyme relies on the fundamental information on the site and conditions of action of the enzyme in the gastrointestinal (GI) tract where it produces most of its beneficial effects.

SITES OF ACTION OF NSP ENZYMES SUCH AS XYLANASES

General Consideration

Animals cannot produce enzymes for breaking down the NSPs that exist in almost all animal feeds. They rely on the micro-organisms in the digestive tract to achieve this. Ruminants have evolved a specific digestive system, where the micro-organisms degrade NSPs in the early absorption site of the gut (the rumen), while monogastric species, such as poultry, expose feed to micro-organisms in the later stages of gut digestion. Comparing the two systems, it is easy to imagine a way in which the enzyme added to a diet of monogastric species can enhance degradation of NSPs in the upper section of the gut (Hotten, 1992).

It has been assumed that a favorable response of supplemental enzymes in the feed is greatly dependent on specific conditions such as the pH of the digestive tract, the time of exposure to enzymes, and the concentrations of the substrates. The endogenous enzymes within the digestive tract are mainly located in the stomach (primarily protein degradation) and the small intestine (protein, lipids and carbohydrates degradation). Therefore, the site of action of exogenous NSP enzymes should be before or at least should coincide with those of the endogenous enzymes.

Feed Passage Pattern in Different Sections of the Gut

Broiler chicks are meal eaters in a comfortable environment with full light and constant access to feed. Under these conditions, they will eat about every 4 hr and drink several times during the 4 h feeding cycle. After eating, a broiler drinks some water, solubilizing part of the feed in the crop. This liquid portion passes into the proventriculus (stomach) and on into the gizzard. Thus, the feed is metered out of the birds' crop. The gizzard is also a metering device, liquefied feed entering the top of the gizzard from the crop, via the stomach, is squeezed by contractions. The emptying time of feed from crop is about 2 h in birds with a normal pattern (*ad libitum*), but highly variable depending on the method of feeding (Classen, 1996; Savage, 1998). A gizzard having received wet mushy feed will contract and expel half the new feed and an equal volume of old feed in 5 to 15 min. Feed entering the duodenal loop passes down the intestine by peristaltic contraction of the intestine. With a normal pattern, feed entering the duodenum exits the intestine in 1.5 to 2 h.

Mean Retention Time (MRT) of Feed

Transit time can be defined as the time it takes the digesta of a meal to pass through the GI tract and can be estimated by the inclusion of a marker in the diet. The time for 50 % of excretion of the marker is an appropriate way to express the rate of feed passage (Ferando et al., 1987; Vergara et al., 1989; van de Klis et al., 1990; van de Klis and Voorst, 1993a). One simple way of calculating transit time is by determining MRT (Coombe and Kay, 1965), based on a steady-state assumption (van de Klis et al., 1990). This implies that the rate of intake of marker is equal to its rate of excretion throughout the day. Using this technology, the MRT for each part of gut can be estimated. The MRT in successive parts of the GI tract

for broilers are listed in Table 3 (Shires et al., 1987; van de Klis et al., 1990 and Danicke et al., 1999). The variation of MRT as indicated by the data in Table 3, especially in that of the crop, might be caused by differences in feed composition, age, and type of birds (Shires et al., 1987). The MRTs in the duodenum plus jejunum are closer to those of the ileum (77 vs. 83 min) and longer than those of the proventriculus plus gizzard (43 min). Theoretically, the longer the MRT is, the better the site is a candidate for enzyme action. In addition, the total amount of feed passing through a site in the gut within a certain time period should also be addressed.

Total Dry Matter Passing through a Section of the Gut

Danicke et al. (1999) recently studied the MRT and the apparent digestibility of DM (dry matter) in each section of the gut (Table 4). The results suggested that the total DM passing through crop, proventriculus plus gizzard, duodenum plus jejunum, and ileum, which could be estimated by the product of the DM and relative MRT, were nearly the same except for the crop which will change according to feeding conditions. The results indicated that crop, proventriculus plus gizzard, and jejunum could be equally important as the main action site for exogenous enzymes.

Action Site of Exogenous Enzymes

Most enzymes of fungal origin show a pH-optimum in a range from 2.5 to 5.0, and therefore they give the best results when used in this pH range. The xylanase produced by *B. subtilis* has a pH-optimum of above 6, which enables the enzyme to act efficiently at the neutral or slightly acidic pH present in the small and large intestine (van de Mierop and Ghesquiere, 1998). Nevertheless, the best sites for the action of NSP enzymes ideally should

TABLE 3. The mean retention time (MRT) in successive segments of the gastrointestinal (GI) tract of broilers as published by several authors (h)

Segment of	Source[1]		
GI tract	1	2	3
Crop	0.52	0.68	0.86
Proventriculus +gizzard	0.65	0.55	0.73
Duodeunum	0.17	0.08	0.22
Jejunum	1.40	1.18	1.65
Ileum	1.62	1.50	0.02
Rectum	0.93	0.43	0.08

[1]Source: (1) Shires et al. (1987), (2) van de Klis et al. (1990), and (3) Danicke et al. (1999).

TABLE 4. Mean retention time (MRT) of digesta and apparent digestibility of dry matter (ADDM), and total passing dry matter (TPDM) in the section of gastrointestinal tract of broilers [1]

Intestinal segment	MRT (h)	ADDM (%)	TPDM[2]
Crop	0.86	-11.7	10
Proventriculus + Gizzard	0.73	-50.7	12
Duodenum	0.22	-25.3	3
Jejunum	1.65	45.7	10
Ileum	2.05	63.2	8
Rectum	0.50	67.4	2

[1]Source: modified from Danicke et al., 1999.

[2]TPDM = DM x MRT / Total MRT, which represents the relative DM passing through each section of gut per day. Feed intake = 78.8 (g/d); Total MRT = 6.89 (h).

be at least before or within the jejunum to overcome the anti-nutritional factors in the digesta. Classen (1996) suggested that the major site of action of NSP enzymes could be in the duodenum plus jejunum of the gut for poultry. Most research has focused on reducing digesta viscosity in the upper part of the small intestine. Boros et al.(1998) suggested that the main site of action for NSP enzymes in broilers occurs in the section of the gastrointestinal (GI) tract below the gizzard on the basis of change in viscosity and reducing sugar level in the different segments of the gut. They demonstrated that most of the viscosity reduction and reducing sugar release occurred in the anterior small intestine. To the contrary, Liebert et al. (1993) demonstrated that 69 to 86 % of exogenous phytase activity was detected in the crop, and that 31 to 38 % of the phytase activity was detected in the proventriculus. No phytase activity was detected in the small intestine. In addition, the optimal pH of phytase showed two response peaks, the highest one was observed at pH 5.0 to 5.5 and the second peak was at pH 2.5 (Irving and Cosgrove, 1974; Shieh et al., 1969; Simons et al., 1990; Nys et al., 1996). This implies that the crop and proventriculus could be the best action sites for phytase. Therefore, the main action site for exogenous enzymes requires further study.

The pH of Digesta

The pH of digesta in different segments of the gut in poultry has been determined (Table 5). These data indicated that an acidic pH persists throughout the GI tract, although neutrality is approached in the lower section of the small intestine and the rectum. The average pH in the crop, proventriculus, gizzard, duodenum, jejunum and ileum was 5.8, 4.7, 5.8, 6.2, and 6.8, respectively. However, different pH values have also been reported; for example, the pH of the gizzard has been reported to be as low as 2.5 (Denbow, 2000). The variation in pH in

TABLE 5. The pH of digesta in different sections of the gastrointestinal tract of chickens

Treatment	Crop	PVC[1]+ Gizzard	Duodenum	Jejunum	Ileum	Source
Corn						van de Klis et al., 1993b
Control	4.8	4.0	5.9	6.0	7.2	
0.5 % CMC	5.0	4.2	5.8	5.8	6.7	
1.0 % CMC	5.1	4.4	5.9	5.8	6.0	
Rye						Boros et al., 1998
Control(-)	6.31	5.66	6.37	6.37	6.28	
Cellulase	6.47	5.11	6.59	6.59	6.96	
Ecozyme	6.47	5.16	6.55	6.55	6.97	
Wheat						
Control(+)	6.30	4.78	6.50	6.50	6.50	
Rye						Danicke et al., 1997
Control	5.79	4.48	4.41	6.07	7.16	
Xylanase	5.85	4.65	4.49	6.01	7.23	
Average	5.8 ±0.67	4.7 ±0.52	5.8 ±0.84	6.2 ±0.32	6.8 ±0.44	

[1]PVC = proventriculus; CMC = carboxymethyl cellulose.

each section of the GI tract is largely influenced by the type of diet, content of NSPs, type of chicken, age of the chicken and the method used to measure pH (e.g., live vs. dead bird). It is important for evaluating the efficacy of feed enzymes to know the pH at the site where an enzyme acts. For example, a pH range from 5.8 to 6.2 may be considered as the optimal pH for evaluating the efficacy of feed enzymes if the main sites of action are located in the duodenum and jejunum. However, fundamental information is still lacking on the location in the GI tract where the exogenous enzymes produce most of their beneficial effects. It is not known, for example, whether the main site of enzyme action in chickens is in the crop, proventriculus, duodenum, jejunum, ileum, or all of the GI tract (Marquardt, 1997).

CURRENT METHODS FOR EVALUATING THE EFFECT OF NSP ENZYMES AND SOME OF THEIR LIMITATIONS

The effects of NSP enzyme supplementation on the performance of chicks fed cereal-based diets are influenced by enzyme source, enzyme dosage, cereal type and variety, amount of cereal in a diet, and even the age of chicks (Jeroch et al., 1995). These effects are generally evaluated by chick performance, metabolizability of energy, digestibility of feed nutrients, and the reduction of viscosity of digesta. Currently, broilers are usually used as the test animal for the assessment of the NSP enzyme effect. Traditionally, the test period frequently only lasted 14 to 21 days, beginning immediately or a few days after hatching. Dramatic effects of NSP enzymes on chick performance during these short-term tests are often observed.

The improved growth of chicks with enzyme supplementation appears to be mainly associated with improvements in energy utilization and nutrient metabolizability (Rotter et al.,

1989a; Carrè et al., 1992). Carrè et al. (1992) demonstrated over a 42-d period that supplemented enzymes improved the metabolizable energy of the diet and thus chick performance. This substantiated the investigation by Choct and Annison (1990) and Annison (1991; 1992) which proved that variations in the content of metabolizable energy of Australian wheats were caused by varying concentration of water-soluble NSP. The improved availability of energy obtained with NSP enzyme when added to diets containing barley, oats, rye or wheat as the grain component has been attributed to improvements in the digestibility of nutrients in general, especially that of fat (Fengler et al., 1988; Carrè et al., 1992; Friesen et al., 1992), but also starch (Annison, 1990, 1992; Carrè et al., 1992) and amino acids (Bedford and Morgen, 1996). Starch digestibility has been reported to account for up to 35 % of the improvement in apparent metabolizable energy (AME) as a result of xylanase supplementation, whilst fat accounts for 35 % and protein for 30 % of the improvement in AME (Carrè et al., 1992; Bedford and Morgen, 1996). The benefits of improved digestion of nutrients can be most likely attributed to the ability of the NSP enzyme to reduce the viscosity of digesta.

Results from studies (Fengler and Marquardt, 1988b; Choct and Annison, 1992), where wheat NSPs were added back to a sorghum-based diet, indicated that the negative effects of viscosity were equally apparent on the digestion of fat. Other studies in our laboratory by Fengler and Marquardt (1988a), and Fengler et al. (1988) confirmed the observation that the viscous arabinoxylans were the factors in the rye grain that were responsible for its antinutritive effects and that partial hydrolysis of the arabinoxylans by an enzyme mixture high in arabinoxylanase activity reduced the viscosity of different mixtures of rye and wheat both

in vitro and in vivo. The reduced viscosity of the diet and digesta was associated with a marked improvement in nutrient retention, particularly fat, and increases in efficiency of feed utilization and growth. Therefore, the effect of NSP enzymes can be further evaluated by its ability to reduce the viscosity of digesta. Bedford and Classen (1992) demonstrated that the improvements with increasing amounts of enzyme in a rye based diet was highly and negatively correlated with a reduction in the viscosity of digesta in both the proximal and distal small intestine. These authors concluded that viscosity alone was responsible for approximately 80 % of the response to enzyme supplementation. In addition, a two-stage in vitro assay for evaluating the effect of NSP enzymes on the viscosity of aqueous extracts of rye-based diets was developed by Bedford and Classen (1993). The in vitro assay could be used to predict the in vivo intestinal viscosity and final weight of birds at 19 d of age (r^2 = 0.66, $p < 0.0001$). In general there is a negative correlation between content of the viscous compounds and the digestion and absorption of all nutrients, the AME of the diet and chick performance, and a positive association between the viscosity of intestinal contents, the incidence of vent pasting, and the production of wet sticky droppings and excreta ammonia. The negative effect of the viscous water soluble NSPs could be neutralized by adding the appropriate mix of enzyme to the diet.

Nevertheless, most of the evaluations, no matter what method that was used, have been based on comparison of the response of NSP enzymes added to a cereal-based diet with that of the control diet (without enzyme addition). However, it is difficult to accurately estimate the effectiveness of feed enzymes (xylanases) in vivo on the basis of their in vitro activities especially if different feeding programs are used. One of the problems encountered by feed-

industry nutritionists is how to select an enzyme preparation that would be most appropriate to their particular products or to determine which of the many different enzyme preparations would be the most effective. In most studies, the amount of enzyme preparations added to a cereal-based chick diet was often selected on the basis of either the levels recommended by the manufacturer or the same amounts of enzyme as determined by an enzyme activity assay (Rotter et al., 1989b; Boros et al, 1998). However, the usefulness of this kind of evaluation could be questioned as discussed previously.

SUMMARY

The efficacy of feed enzymes such as xylanases can be defined as the ability of enzyme to improve chick performance or to reduce dietary viscosity when added to a cereal-based diet, and it should be a relatively constant value when other variables are held constant. However, many factors may influence the evaluation of the efficacy of enzymes. First of all, the target substrates in cereals have a complex structure with variable physical and chemical properties, which influence the interaction or reactivity of the substrate with the enzyme. Second, most enzyme preparations that are available come from different strains of fungi and bacteria. They vary considerably in pH optimum, substrate preference and temperature optimum, which in turn determines the ability of an enzyme to catalyze the hydrolysis of a specific substrate. Third, the major site of action of enzymes in the GI tract needs to be identified in order to develop effective in vitro methods for evaluating their efficacy. In addition, the mechanism by which feed enzymes are able to produce their beneficial effects needs to be considered. Currently, it is not possible to compare efficacy of the many different feed enzymes that are

marketed as there is no standard assay, and as a result it is not possible to establish an accurate relationship between in vitro activity values as reported by the manufacturer and resulting efficacy of the enzyme when added to poultry diets. Therefore, the successful development of a method, that can be used to evaluate the efficacy of a feed enzyme and predict animal response, would greatly assist the feed industry in the selection of efficient enzyme preparations and would stimulate the improvement of the quality of feed enzymes. Therefore, this research should provide both basic and practical information on the use of enzymes as feed additives which in turn will further enhance their use in the feed industry.

HYPOTHESIS AND OBJECTIVES OF THE RESEARCH PROPOSAL: ESTIMATING THE EFFICACY OF FEED ENZYMES

Dose Response Studies

Dose response studies are required to establish the nature of the response obtained per increment of an enzyme when added to a specific type of diet. Friesen et al. (1991) carried out two dose-response experiments using different enzyme concentrations to determine the level of a crude enzyme preparation required to obtain maximum growth performance in broiler chicks fed a 60% rye-based diet. The results clearly indicated that a hyperbolic dose-response curve was obtained. Another study also confirmed this relationship (Marquardt et al., 1994). In this study, a dose-response experiment established the effect of enzyme supplementation on the performance of Leghorn chicks when added to diets containing high amounts of rye, barley, wheat or corn. The result clearly demonstrated that the effectiveness of an enzyme preparation was different when added to the different cereal-based diets but that

the dose-response curves for all diets exhibited a saturation response pattern except for the corn-based diet which did not show a response to enzyme treatment. Studies by Bedford and Classen (1992) demonstrated that the effectiveness of an enzyme was also different when added to diets containing different levels of rye. They investigated the effect of rye level (0, 20, 40 and 60% in a diet) and xylanase inclusion (0, 1, 2, 4, 8, 16 g/kg) in the diet on chick performance. The results demonstrated that the effect of the enzyme added to the rye-based diets was increased with increasing levels of rye in the diet. Also, the dose-response curves yielded a typical hyperbolic saturated pattern for all levels of rye in the diets. In addition, increasing concentrations of enzyme produced a corresponding hyperbolic decrease in the intestinal viscosity of digesta. It is concluded, on the basis of this review, that the hyperbolic response pattern in chicks fed increasing concentrations of enzymes is a general pattern, and the magnitude of the response reflects, among other things, the ability of the enzyme to act on its target substrates.

Log-linear Model and Hypothesis

The hyperbolic curve of dose response reflects a common biological phenomenon, the Law of Diminishing Returns. The curve could be generated by several non-linear models. They include polynomial, exponential, logarithmical models (Almquist, 1952; SAS, 1988), or other models such as a segmented model or logistic model (Remmenga et al., 1997). However, in the feed enzyme field, only a few studies applied some of these models to data. For example, in the study of Friesen et al. (1991), the exponential model was applied to estimate the maximum effective dose required of a feed enzyme added to a 60% rye-based diet. In another study, Hesselman et al. (1982) applied the polynomial model (linear and quadratic model) to

predict the level of a β-glucanase preparation required to maximize the productive value of broiler chick diets containing barley harvested at two stages of ripeness. Almquist (1952) demonstrated that a logarithmic method for evaluating data was extremely useful in many diversified applications of biology since these relationships were merely expressions of the Law of Diminishing Returns. A particularly valuable feature of the logarithmic method of expressing the relationship between intake and biological response is the fact that it provides a simple but accurate estimation of the slope of the response line. The slope of the line in the study was mainly associated with the magnitude of the conversion rate constant for a provitamin to vitamin in the intestinal wall. The constant was dependent upon many dietary and physiological factors in an absolute sense, but was relatively constant within any one bioassay. Therefore, we hypothesize that the logarithmic method could be used to predict the response of chicks to a feed enzyme as shown in equation 1:

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

Where, Y = response of an enzyme, X = amount of enzyme added to a diet, A = response without enzyme addition, and B = slope of the line. In addition, the enzyme added to a diet, in a manner similar to the conversion of a provitamin into a vitamin, would convert a nutrient from unavailable, because of anti-nutritive NSP in the digesta, to available through the degradation of an anti-nutritive NSP. The magnitude of the conversion should reflect the ability of an enzyme to produce beneficial effects (i.e., the efficacy of a feed enzyme). The ability or efficacy of a feed enzyme in turn could be readily obtained from the slope of the log-linear model. Although Almquist (1952) has demonstrated that there is an accurate linear logarithmic relationship between dietary concentrations of certain nutrients and their

corresponding uptake or metabolic response, the nutrition community has essentially ignored these important observation for about a half century. Perhaps one of the reasons is that suitable equations were not developed and computations were time consuming. The current availability of powerful computers has eliminated the second factor.

Objectives of the Research:

The objectives of this research are:

1) to determine if the response of chickens to dietary enzymes can be accurately predicted using a log-linear model equation,

2) to determine the utility of specific parameters of the model including its ability to assess the efficacy of an enzyme when added to the diet (i.e., the slope of the model equation),

3) to adapt the model to a least-cost analysis that will provide a basis for estimating economic return per unit of enzyme added to a diet,

4) to develop a simple in vitro dietary viscosity assay for estimating the amount of enzyme that needs to be added to a cereal-based diet, and

5) to demonstrate that the activity values of diets containing different amounts of enzyme as determined in vitro when used in conjunction with performance parameters as obtained from the log-linear model can be used to accurately assess the efficacy of an enzyme.

MANUSCRIPT 1

A SIMPLE MODEL FOR PREDICTING THE RESPONSE OF CHICKS TO DIETARY ENZYME SUPPLEMENTATION

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ABSTRACT Two experiments with Leghorn chicks and data from five publications were analyzed to determine whether a simple general equation could be used to predict the relationship between the amount of a crude enzyme added to a diet and chick performance. The maximum improvements in weight gain and feed to gain ratio in Leghorn chicks fed rye diets containing different concentrations of enzymes were as high as 61 and 42%, respectively. Regression analyses demonstrated that there was a high linear correlation ($r^2 > 0.91$, $P < 0.05$) between the concentration of the enzyme when transformed into its logarithmic value and weight gain or the feed to gain ratio. The general prediction equation was: $Y=A + B \log X$ where Y is the performance value (ie., weight gain, grams), A is the intercept (*y*-axis), B is the slope of the line (change in performance per log unit of enzyme in the diet) and X is the amount of enzyme in the diet. The slope of the line provides an index of the overall efficacy of the enzyme treatment. The log-linear model shows that for every ninefold increase in amount of enzyme in the diet (i.e., when the amount was increased to 10 times the starting amount), there was only a doubling of improvement in chick performance. High correlations (r^2 values) were also obtained when data from the literature were analyzed. The equation was applicable to different classes and ages of poultry fed diets containing rye, wheat, barley, or lupins. These studies demonstrate that there is a linear relationship between the amount of enzyme added to the diet, when expressed as a logarithmic value, and the corresponding performance of chicks.

(*Key words*: Enzymes, Poultry, Models)

INTRODUCTION

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 Aman 1986; Friesen et al., 1992; Marquardt et al., 1994), oats (Friesen et al., 1992), rye (Fengler and Marquardt, 1988b; Fengler et al., 1988; Bedford and Classen, 1992; Friesen et al., 1991, 1992; Marquardt et al., 1994), and wheat (Fengler et al., 1988; Friesen et al., 1991; Marquardt et al., 1994), and in 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 model has been developed that can simply predict their effects on animal performance. The objective of this study was to determine whether the response of animals 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) that are efficiently hydrolysed by xylanases (Fengler and Marquardt 1988a; Fengler et al., 1988; Marquardt et al., 1994). Wheat was used to replace rye because it has much lower levels of these viscous compounds. Data from the literature were also analyzed.

MATERIALS AND METHODS

Source of Cereals and Enzymes

Wheat (variety, Katepwa) and rye (variety, Prima) were from local sources. Two enzyme preparations, RM1[1] and NQ[2] were used in this study. They were high in xylanase (3,450 IU/g and 7,700 IU/g, respectively), and ß-glucanase (900 IU/g and 3,300 IU/g, respectively) activities. The activity of these enzymes was assayed by Finnfeeds International using the 3,5 dinitrosalicylic acid reducing sugar method for xylanase and ß-glucanase. ß-glucan (pH 5.0) was used as the substrate for the assay endo-1, 4-ß-glucanase (Miller, 1959) and xylan (pH 5.3) as the substrate for xylanase (Bailey, 1988). All enzyme preparations were also assayed for xylanase activity using the azo-dye method of McCleary (1992) using arbinoxylan as the substrate. These values were 389 and 778 U/g for RM1 and NQ, respectively and were used in the regression analysis study.

Diets

The diets were formulated as outlined in Table 6 and meet the National Research Requirements for Leghorn chicks (NRC, 1994). In Experiment 1, different amounts of RM1 (0, .25, .75, 2.25, 6.75, and 20.25 g/kg) and NQ (0, .1, .3 .9, 2.7, and 8.1 g/kg) were added to the rye diet for a total of 12 different treatments. In Experiment 2, different concentrations of NQ and RM1 were added to the rye diet for a total of six treatments. The treatments, numbered consecutively from 1 to 6, were as follows: no enzyme, 1 g/kg NQ, 20 g/kg NQ, .33 g/kg RM1, 1 g/kg RM1, 3 g/kg RM1.

Chick Management

1-d-old Single Comb White Leghorn cockerels were fed a commercial starter diet for a 7-d

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[1]Finnfeeds International Ltd., Wiltshire, UK SN8 1XN.

[2]Nutri-Quest, Chesterfield, MO 63017.

TABLE 6. Composition and calculated analysis of the experimental basal diets, as-fed basis (Experiments 1 and 2)[1]

Ingredient, g/kg	Experiment 1	Experiment 2
Rye (9.4% crude protein)[2]	600	600
Wheat (15.1% crude protein)[2]	82.5	85.5
Chromic oxide	3	0
Soybean meal (45% crude protein)	245	245
Vegetable oil	29	29
Limestone	12	12
Calcium biphosphate	13.5	13.5
Mineral mix[3]	5	5
Vitamin mix[3]	10	10
Calculated composition		
MEn., MJ/kg	12.40	12.44
Crude protein, %	18	18
Lysine, %	1.06	1.06
Methionine and cystine, %	0.66	0.66
Ca, %	0.81	0.81
P available, %	0.4	0.4

[1]The crude enzymes were added to the diet at the expense of rye.

[2]The variety of rye and wheat were Prima and Katepwa, respectively.

[3]The vitamin mix and mineral mix supplied the following (per kilogram of diet): vitamin A, 8,250 IU; vitamin D_3, 1,000 IU; vitamin E, 10.9 IU; vitamin B_{12}, 0.0115 mg; vitamin K, 1.1 mg; vitamin B_2, 5.5 mg; d-Ca pantothenate, 11.0 mg; niacin, 53.3 mg; folic acid, 0.75 mg; d-biotin, 0.25 mg; choline chloride, 1,020 mg; Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.18 mg and NaCl, 2.5 g.

pre-experimental period and then after 4 h of food starvation were randomly distributed in groups in such a way that all groups had the same average weight. The experimental diets were fed to birds from 7 to 21 d of age. The 7-d average weights of the chicks were 99 and 95 g for Exp.1 and 2, respectively. Chick weight and feed consumption were recorded 4 h after removal of feed at 14 and 21 d of age. In the course of the experiments, chicks were kept in the same Petersime batteries with constant light and heat and had free access to water and feed. In each of the two experiments there were six replicates per treatment and six birds per replicate (cage). The research proposal was reviewed and approved by the local animal care committee and followed the guidelines of the Canadian Council of Animal Care.

Analyses of Data

Data from Exp. 1 and 2 were analyzed according to SAS (1988). A completely randomized design was used with the experimental unit being a cage unit. Data were analyzed by ANOVA to determine significance of main effects. Duncan's multiple range test and orthogonal polynomial contrasts were used for multiple comparison of means. The means of the data were also subjected to regression analysis using linear, quadratic and cubic polynomials. In most of these analyses the amount of enzyme or its activity was transformed into its logarithmic value. The data were plotted using Sigma Plot (Kuo and Norby, 1992).

RESULTS AND DISCUSSION

General Observations (Experiment 1)

Analysis of variance of the data demonstrated that there were no interactions between enzyme source and enzyme amount for weight gain ($P > .05$, Table 7). The interaction was

significant for feed to gain ratio only in wk 2 ($P > .05$, Table 7) and was considered to be of minor importance because it was responsible for only 3% of the total variance when the sum of squares were partitioned. Except for weight gain during wk 1, there was no difference in the response of chicks to the two enzyme preparations ($P > .05$). The data for the two enzymes was therefore pooled (Table 7). There was, however, a marked effect of the amount of enzyme in the diet on the performance of Leghorn chicks for all time periods ($P < .001$). The overall improvement in weight gain and feed to gain ratio with enzyme addition over the 2-wk period were 28 and 12%, respectively ($P < .05$). Response in weight gain and feed to gain ratio to enzyme treatment was also greater during wk 1 (51 and 22%, respectively, $P < .05$) than in wk 2 (16 and 5%, respectively, $P < .05$). The response to enzyme treatment had linear ($P > .001$), quadratic ($P \leq .017$ to .001), and, in some cases, cubic ($P < .001$, expect for data from wk 2) components. This suggests that the response was not directly proportional to the amount of enzyme added. For example, in wk 1 the addition of 88 U of enzyme/kg of diet to the control diet increased weight gain by 23% ($P < .05$), whereas increasing the amount of enzyme from 2,363 to 7,090 U/kg diet (an increase of 4,727 U) improved weight gain by only additional 7% ($P < .05$). Similar trends were obtained for all other comparisons. Overall, the results indicate that the response to enzyme treatment was not proportional to amount of enzyme added to the diet and that it was much greater in wk 1 than in wk 2 of the study.

Prediction Model from Curve Fitting Analyses (Experiment 1)

The data from Experiment 1 were subjected to different types of regression analysis (Table 8). The amount of enzyme added to the diet was generally not significantly correlated ($P >$

TABLE 7. The performance of Leghorn chicks fed a rye-based diet containing different amount of two enzyme preparations (Experiment 1)[1]

Enzyme activity in diet, U/kg	Weight gain/g, 6 birds)[2]			Feed to gain ratio[2]		
	Wk 1	Wk 2	Wk 1+2	Wk 1	Wk 2	Wk 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]
2363	276[b]	390[ab]	666[ab]	2.15[cd]	2.18[c]	2.16[d]
7090	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
Source of variation	Probabilities					
Enzyme source (ES)	0.023	0.93	0.37	0.17	0.41	0.5
Enzyme activity (EA)	0.001	0.001	0.001	0.001	0.001	0.001
ES x EA	0.11	0.65	0.76	0.48	0.024	0.48
Type of response due to enzyme activity						
Linear	0.001	0.001	0.001	0.001	0.001	0.001
Quadratic	0.001	0.017	0.001	0.001	0.001	0.001
Cubic	0.001	0.43	0.001	0.001	0.4	0.001

[1]The values represent weight gains for the treatment group (six birds). The average initial 7-d weight of chicks in each treatment was 99 g, n = 6 birds per replicate. Each treatment consisted of six replicates. A total of 12 diets were fed: six containing different amounts of RM1 and six different amounts of NQ. The xylanase activity in the two preparations was 389 U/g RM1 and 778 U/g for NQ. The amounts of RM1 added to the diets were 0, .25, .75, 2.25, 6.75, and 20.2 g/kg to give final activity values of 0, 97, 292, 875, 2,626 and 7,877 U/kg, respectively. The amounts of NQ added to the diets were 0, .1, .3, .9, 2.7, and 8.1 g/kg to give final activity values of 0, 79, 233, 700, 2,100, 6,302 U/kg, respectively.

[2]Means in each column not showing a common letter (a to e) are significantly different ($P <$ .05).

[3]Mean weight gains for wk 1 for RM1 and NQ were 254 and 263 g/6 birds, respectively.

[4]Feed to gain ratio values for RM1 for the interaction from the lowest to the highest amount of added enzyme were 2.30, 2.30, 2.28, 2.21, 2.18, and 2.12; corresponding values for NQ were 2.31, 2.24, 2.25, 2.26, 2.18, and 2.23.

TABLE 8. The relationship between chick performance and units of enzyme activity added to the diet (Experiment 1)[1]

	Weight gain						Feed to gain ratio					
	Wk 1		Wk 2		Wk 1 + 2		Wk 1		Wk 2		Wk 1 + 2	
Type of regression analyses	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[2]	0.97	0.001	0.91	0.003	0.99	0.001	0.92	0.003	0.89	0.005	0.99	0.001
Log-quadratic[3]	0.98	0.001	0.98	0.002	0.99	0.001	0.98	0.004	0.94	0.01	0.99	0.001
Log-cubic[3]	0.98	0.03	0.98	0.02	0.99	0.003	0.98	0.03	0.96	0.06	0.99	0.004

[1]See Table 2 and Material and Methods for further details. Pooled data for both enzyme types (RM1 and NQ) were used in the analyses.

[2]The assigned value (the ε value) for the diets without enzyme addition was 3.2 U/kg diet.

[3]The differences between the first (log-linear) and second (log-quadratic), and the first or second and third (log-cubic)-order parameters were not significant ($P > .05$).

.05) with chick performance during wk 1 when the data were subjected to linear, quadratic, or cubic regression analyses. The exception was for weight gain and feed to gain ratio in wk 2 ($P \leq .06$), a time period where the response to enzyme treatment was the least (Table 7). However, when the dietary enzyme concentration data were converted into their logarithmic values and subjected to linear regression analysis, all of the log-linear values were significant ($P \leq .005$), with all regression values (r^2) being greater than .88. The r^2 values for the combined wk 1 and 2 data for weight gain and feed to gain ratio were .99 ($P < .001$). Although some numerical improvements were obtained using quadratic and cubic terms, these were generally not significant improvements ($P < .05$).

Not only did the log-linear model have the highest r^2 and lowest P values among the models tested, but it is also a prediction equation (Table 9) that is simple to interpret with the performance of chicks being a linear function of the logarithm of the amount of the enzyme added to the diet. The model predicts that a ninefold increase in enzyme concentration in the diet (i.e., an increase to 10 times the starting amount) will result in a onefold (100%), not a ninefold, improvement in animal performance (i.e., an increase in enzyme from 10 to 100 relative concentration units 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 while much larger amounts are required for each additional incremental improvement.

In regression analyses, the selection of an appropriate intercept value for diets that do not contain any added enzyme (zero enzyme), however, must be made as a value for the logarithm of zero cannot be calculated. Because the value for the logarithm of zero cannot

TABLE 9. Regression equations describing the linear relationship between the performance of Leghorn chicks and the logarithmic content of enzyme in the diet (Experiment 1)[1]

Parameter	Equation[2,3]	Probability		Residual SD
		Intercept (A)	Slope (B)	
Body weight gain (g/6 birds)				
Wk 1	Y = 185 + 29 log X	0.001	0.001	7
Wk 2	Y = 331 + 16 log X	0.001	0.003	7
Wk 1 + 2	Y = 517 + 45 log X	0.001	0.001	3
Feed to gain ratio				
Wk 1	Y = 2.67 - .17 log X	0.001	0.003	0.07
Wk 2	Y = 2.33 - .04 log X	0.001	0.005	0.02
Wk 1 + 2	Y = 2.46 - .09 log X	0.001	0.001	0.01
General equation[3]	Y = A + B log X			

[1]Data was calculated from pooled data from the two enzyme (RM1 plus NQ). See Table 1 to 3 and Materials and Methods for further detail.

[2]The activity values (ε) that was used for the diet with no enzyme supplementation was from 3.2 U/kg diet. See text for the derivation of this value. Substitution of this value into the equation will provide an estimate of the performance of chicks in the presence of nearly a zero amount of enzyme. The actual values without enzyme addition are given in table 2.

[3]The equations predict the performance of chicks during wk 1, wk 2, and wk 1 + 2 for diets containing different amounts of enzyme (U/kg diet). The values Y = predicted performance [weight gain (g /6 birds) or feed to gain ratio] of chicks during the different time periods; X = amount of enzyme (U/kg diet) added to the diet; B = the slope of the line [weight gain (grams/six birds) or feed to gain ratio per log unit of added enzyme per kg diet]; and A = intercept of the line (grams or grams/gram). The intercept, A, is the estimated performance of chicks fed very low levels of enzyme (the ε value).

be calculated, any value close to the zero value can be substituted for the zero value (Cody and Smith, 1991). However, the substitution of various small values for the amount of enzyme can result in r^2 values that are quite different. Under such conditions a value for zero should be selected that is close to the zero value and yields the largest r^2 and lowest error mean square values. The value used to represent the diet with zero enzyme supplement was 3.2 U/kg diet (see footnotes of Table 9).

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

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

where Y = the performance value (i.e., weight gain, grams), A = the intercept (*y* axis), B = the slope of the line, and X = the amount of enzyme (grams or units per kilogram diet). Assume Yo = the weight gain over a certain period of time without added enzyme. Under such conditions X = Xo = O and Equation [2] is as follows:

$$Y - Yo = A - Yo + B \log X \qquad [2]$$

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:

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

When X = Xo = 0, the total amount of enzyme added in diet is zero. Under such conditions, Y = Yo and Equation [4] equals:

$$A - Yo + B \log \varepsilon = 0 \qquad [4]$$

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

The value ε, is derived from Equation 5 for each set of data and used as a substitute for

zero value of enzyme concentration. Under such conditions, an ε value that yields the highest r^2 value and the corresponding smallest error (sums of squares) can be calculated. Using these assumptions, regression equations were obtained that predicted weight gains and feed to gain ratios for chicks of different ages (Table 9).

A comparison of the actual performance values obtained with the two enzymes for the combined results of wk 1 and 2 of Experiment 1 with those predicted from the equation demonstrated that the two sets of values, in all cases, were nearly the same; the maximum differences between them was less than 1% (Figure 6). These differences are relatively small compared to the response obtained with enzyme supplementation. The inset of Figure 6 showed that there was a linear change in weight gain and feed to gain ratio when the concentration of enzyme was plotted on a logarithmic scale. The intercept (A) of the equation represents the weight gain or the feed to gain ratio for a preselected amount of enzyme [the ε value (log 3.2)], whereas the slope of the curve (B, weight gain or feed to gain ratio per log unit of enzyme) provides a basis for evaluating efficacy of an enzyme preparation (see footnote in Table 9). Data from the slopes of the equation as shown in Table 9 suggested that the response to enzyme treatment was more effective during wk 1 than during wk 2. The weight gain per log unit of enzyme added per kilogram of feed was 29 g for wk 1, 16 g for wk 2, and 45 g for wk 1 + wk 2 (29 + 16 g). Corresponding B values for the feed to gain ratio values were −.17, −.04, and −.09.

General Applicability of Model (Experiment 2 and Literature Data)

The general applicability of the model was tested using data from a second experiment (Table 10, Experiment 2) and those from the literature. The objective of these analyses was

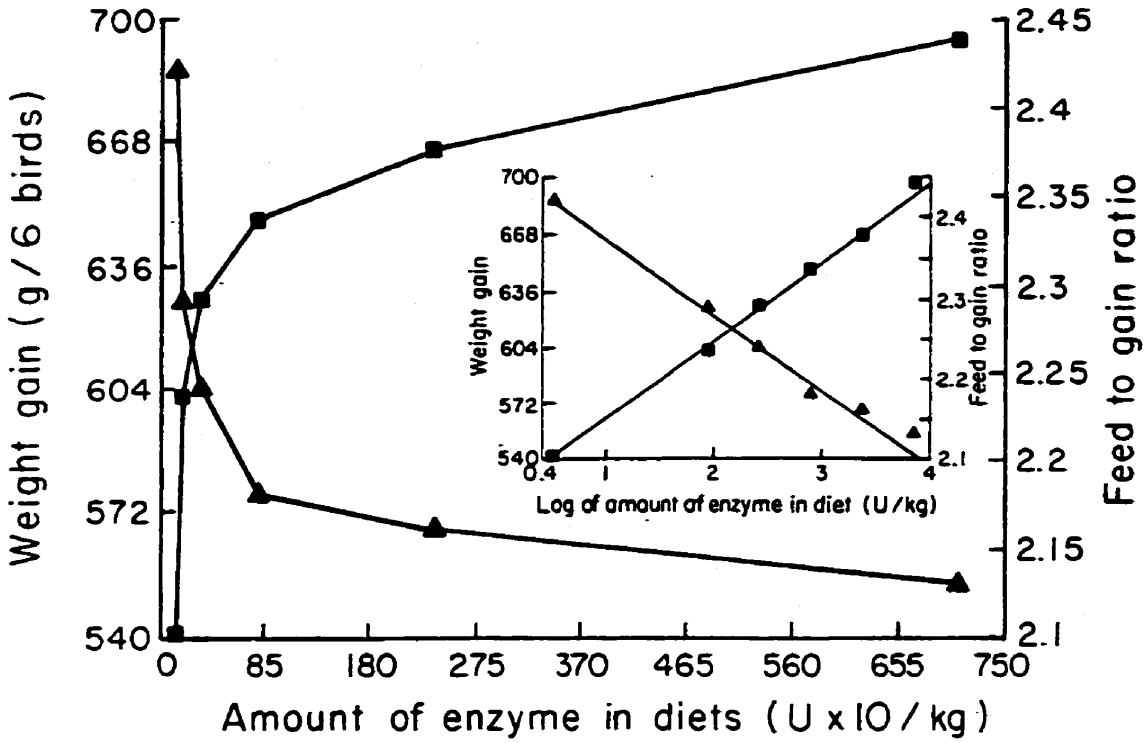

FIGURE 6. The predicted relationship between chick performance during the 1st plus 2nd wk of experiment, and the amount of crude xylanase added to a rye-based diet as determined from the equation Y= 517 + 45 logX (r^2 = 1.00, residual SD = 3 g) or Y = 2.46 - .09 logX (or r^2 = .99, residual SD = .01) where X = units of enzyme in the diet and Y = weight gain (g) or the feed to gain ratio, respectively, (Table 9, Experiment 1). Mean experiment values for weight gain (■) and feed to gain ratio (▲) are also shown. Inset figure represents the same data except the amounts of enzyme have been transformed into their logarithmic values.

TABLE 10. The performance of Leghorn chicks fed a rye-based diet containing several combinations and concentrations of four crude enzyme preparations (Experiment 2)[1]

		Weight gain (g per 6 birds)[2]			Feed to gain ratio[2]		
Diet	Enzyme, g/kg diet	Wk 1	Wk 2	Wk 1+2	Wk 1	Wk 2	Wk 1+2
1	No enzyme (0 U)[2]	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)	281	384	664	2.18	2.1	2.14
4	.33 RM1 (128 U)	210	320	530	2.51	2.23	2.34
5	1 RM1 (389 U)	231	336	567	2.38	2.25	2.3
6	3 RM1 (1,167)	237	360	597	2.38	2.15	2.24
Pooled SEM		16	22	32	0.13	0.04	0.08
Linear contrast (% increase or decrease relative to first comparison[3])							
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. .33 RM1)		21*	-2	6	-14*	3	-3
1 vs. 5 (0 vs.1 RM1)		33*	3	13*	-18*	4	-5
4 vs. 5 (.33 RM1 vs. 1 RM1)		10*	5	7	-5	1	-2
4 vs. 6 (.33 RM1 vs. 3 RM1)		13*	12*	13*	-5	-4	-5
5 vs. 6 (1 RM1 vs. 3 RM1)		2	7	5	0	-5	-3

[1]The average initial weight of chicks in each treatment was 95 g, n = 6 birds per replicate. Each treatment consisted of six replicates. The xylanase activities of RM1 and NQ were 389 and 778 U/g. See Materials and Methods for further detail on the enzyme preparations.

[2]Values in brackets indicate units of xylanase added per kilogram of diet.

[3]Contrats with superscripts differ, * $P < .05$. The experiment-wise Type I error rate was set at $\alpha = .05$ by using a comparison-wise Type I error rate of .05/7 where there were 7 a priori comparisons among the means (SAS, 1988).

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. The second experiment involved the use of several different concentrations of the two enzyme preparations, each preparation having different xylanase activity (Table 10). Linear contrast comparisons demonstrated that the trends for both enzymes were similar to the results obtained in Experiment 1. In general, the response to enzyme treatment was greater in wk 1 than in wk 2, and the response to enzyme treatment was not directly proportional to the amount of enzyme added in the diet. For example, the maximum improvement in weight gain obtained in wk 1 with enzyme addition was 61% ($P < .05$) (0 vs. 20 U/kg NQ), whereas the corresponding improvement in wk 2 was only 17% ($P < .05$). Likewise, the addition of a small amount of enzyme (0 to 1 g of NQ/kg diet) improved weight gains by 42% ($P < .05$), whereas the addition of much greater amounts of enzyme (1 to 20 g of NQ) to the diet produced an additional incremental improvement of only 14% ($P < .05$). Similar trends were observed with the feed to gain ratio and RM1 for both response variables.

Regression analysis of the data from Table 10 is outlined in Table 11, data sets 1 to 3 (NQ) and data sets 4 to 6 (RM1). The results demonstrated that high r^2 values (.84 to .999) were obtained during wk 1 and during wk 1 + 2 of the experiment but not during wk 2. The slope of the lines (B) shows that the response to enzyme was also high in wk 1 and wk 1+2 but not in wk 2. This pattern of response can be attributed to a high degree of response to enzyme supplementation of Leghorn chicks during wk 1 but not wk 2. Overall, the data demonstrates that it is possible to evaluate the response to xylanase supplementation in chicks at different ages even though the enzyme is from different sources.

Data from the literature were used to determine whether a similar relationship was also obtained between the log of the amount of enzyme added to the diet and chick performance (data sets 7 to 19, Table 11). Among the 13 comparisons, nine yielded r^2 values for weight gain of greater than .91 with all but one comparison being greater than .77. Regression analysis of the feed to gain ratio also yielded similar trends.

In addition, high r^2 values were obtained under different feeding conditions (e.g., when enzyme was added to different cereals [rye, wheat and barley] and a grain legume [lupins]), with different enzyme preparations that were high in xylanase and ß-glucanase activity, with different types of enzymes (ß-galactosidase, ß-glucanase and xylanase), with different concentrations of two cereals in the diet (wheat and rye), and with different age and type of chickens (Leghorn vs. broiler).

Prediction of Response to Enzyme Treatment in Chicks Fed Diets Containing Variable Amounts of Two Cereals

The objective of this study was to determine if the model could be used not only to predict the response of a given diet to different amounts of one enzyme but also to determine whether this model could be extended to include different amounts of any two dietary components such as cereals. To achieve this goal, an experiment must be performed in which different amounts of an enzyme are added to diets containing different proportions of two cereals. Such an experiment was conducted by Bedford and Classen (1992). They fed four different concentrations of rye each with six different concentrations of enzyme (xylanase from *Trichoderma longibrachiatum*) to broiler chicks from 1 to 19 d of age in a 4 x 6 factorial arrangement of treatments. The diets consisted of the following proportions of rye and wheat:

TABLE 11. Prediction of goodness of fit between chick performance and enzyme concentration as obtained from several different studies

Data set[1,2]	Diet (%)[3]	Enzyme type[4]	Chick type[5]	Age d[5]	Weight gain[1]					Feed to gain ratio[1]					References
					r^2	P[6]	g	P[7]	g + g/kg	P[7]	r^2	P[6]	g / g	P[7]	g/g + g/kg	P[7]	
1	Rye (60)	Xylanase	L	7-14	.999	*.01*	248	*.002*	24.8	*.01*	.99	*.05*	2.4	*.007*	-.175	*.05*	Experiment 2, Diets 1,2,3
2	Rye (60)	Xylanase	L	14-21	.81	*.30*	358	*.02*	11.9	*.29*	.3	*.60*	2.1	*.009*	-.010	*.63*	Experiment 2, Diets 1,2,3
3	Rye (60)	Xylanase	L	7-21	.98	*.08*	606	*.01*	36.8	*.10*	.99	*.06*	2.38	*.003*	-.064	*.06*	Experiment 2, Diets 1,2,3
4	Rye (60)	Xylanase	L	7-14	.97	*.02*	225	*.001*	17.4	*.02*	.97	*.02*	2.14	*.001*	-.156	*.02*	Experiment 2, Diets 1,4,5,6
5	Rye (60)	Xylanase	L	14-21	.36	*.40*	340	*.001*	6.5	*.40*	.07	*.70*	2.23	*.001*	-.008	*.74*	Experiment 2, Diets 1,4,5,6
6	Rye (60)	Xylanase	L	7-21	.84	*.08*	565	*.001*	24.2	*.08*	93	*.04*	2.44	*.001*	-.045	*.04*	Experiment 2, Diets 1,4,5,6
7	Rye (60)	Xylanase	L	1-14	96	*.001*	122	*.001*	29.3	*.001*	94	*.001*	2.20	*.001*	-.496	*.001*	Friesen et al., 1992
8	Rye (0)	Xylanase	B	1-19	.49	*.48*	436	*.001*	10.5	*.12*	.43	*.16*	2.29	*.001*	-.036	*.16*	Bedford & Classen, 1992
9	Rye (20)	Xylanase	B	1-19	.92	*.002*	420	*.001*	18.8	*.002*	.65	*.05*	1.75	*.001*	-.052	*.05*	
10	Rye (40)	Xylanase	B	1-19	.94	*.001*	419	*.001*	36.1	*.002*	62	*.06*	1.56	*.001*	-.058	*.06*	
11	Rye (60)	Xylanase	B	1-19	.96	*.001*	394	*.001*	54.4	*.001*	95	*.001*	1.64	*.001*	-.205	*.001*	
12	Rye (64)	Xylanase	B	7-21	.999	*.001*	123	*.001*	8.8	*.001*	.997	*.002*	2.01	*.001*	-.090	*.002*	Marquardt et al., 1994
13	Wheat (68)	Xylanase	L	7-21	.92	*.04*	132	*.001*	2.3	*.04*	99	*.007*	1.91	*.001*	-.028	*.007*	
14	Barley (66)	β-Glucanase	L	7-21	.96	*.02*	133	*.001*	6.6	*.02*	98	*.06*	1.83	*.001*	-.038	*.06*	
15	Barley (65)	β-Glucanase	B	14	.8	*.10*	230	*.002*	15.3	*.10*	.88	*.01*	1.38	*.001*	-.041	*.01*	Hesselman et al, 1982
16	Barley (65)	β-Glucanase	B	14	.99	*.006*	234	*.001*	14.0	*.006*	.98	*.04*	1.36	*.001*	-.032	*.04*	
17	Barley (65)	β-Glucanase	B	21	.86	*.07*	480	*.001*	37.4	*.07*	.85	*.08*	1.74	*.001*	-.064	*.08*	
18	Barley (65)	β-Glucanase	B	21	.96	*.02*	484	*.001*	32.6	*.02*	.94	*.03*	1.73	*.001*	-.054	*.03*	
19	Lupin (50)	β-Galactosidase	L	7-21	.78	*.10*	120	*.001*	6.8	*.10*	.92	*.04*	2.00	*.001*	-.105	*.04*	Brenes et al., 1993

[1]Results for data set 1 to 6 were obtained from Experiment 2. The basal diet contained rye grain with different amounts of two

types of enzymes in each data set. Diets in data set 1 to 3 each contained NQ at different concentrations; diets in data set 4 to 6 each contained different concentrations of RM1. The diet number, units of enzyme activity per kilogram diet and type of enzyme were as follows: 1, 0 U; 2, 778 U NQ; 3, 15,560 U NQ; 4, 128 U RM1; 5, 389 U RM1; and 6, 1,167 U RM. The xylanase activity of RM1and NQ as determined by the method of McLeary (1992) were 389 and 778 U of xylanase per gram. See Materials and Methods and Table 5 for further information.

[2]Data for data sets 7 to 19 were from the literature.

[3]The value in brackets represents percentage of cereal or other constituents that were in the diets.

[4]The amount of enzyme added to the diets were expressed as gram per kilogram of feed except for diet 8 to 11, where enzyme concentrations were expressed as a percent value.

[5]Chick types were: L, leghorn, B, broiler. Age refers to age of chick during the test period.

[6]Enzyme activity values were converted to their logarithmic value prior to regression analysis. In this study the relationship (r^2) between the log of the amount or percentage of enzyme in the diet and chick performance was determined by regression analysis. The A values represents the intercept and the B value is the slope of the line for the equation $Y = A + B \log X$ (see Equation [1] in the text. The ε values (see Equation [5] in the test) was .001 d enzyme/kg diet and is the value used for the treatment with no enzyme addition.

[7]The units for weight gain are grams (A value) and grams weight gain per gram enzyme added to 1 kg of diet (B value) for diet 1 to 7 and 12 to 19. Amount of enzyme added to diets 8 to 11 were expressed as a percentage of the diet and therefore percent is used in place of grams of enzyme per kilogram of diet. The corresponding units for the feed:gain ratio were gram/gram (A value) and grams/gram per gram of enzyme added to 1 kg of diet (B value). The weight gain values represents weight gains per six birds for data sets 1 to 6 and weight gain per bird for all other data sets.

00, 0:60; 20, 20:40; 40, 40:20; and 60, 60:0, respectively. The amounts of enzyme added to each of different diets were 0, .1, .2, .4, .8, and 1.6%. The prediction equations as derived from Equation [1] for each diet for weight gain and the feed to gain ratio, as obtained from an analysis of the mean data from Bedford and Classen (1992), are summarized in Table 11 (data sets 8 to 11).

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

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

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 the above model is shown in Figure 7. Similar prediction equations can be generated for the feed to gain ratio or any other variable that fits the model.

An important observation from data presented in Figure 7 is that the amount of enzyme required to obtain a given level of performance is much greater when the concentration of the antinutritive factor (i.e., arabinoxylan) in the diet is high. For example, the amount of enzyme (percentage of diet) required to obtain a weight gain of 400 g for chicks fed 20, 40, and 60% rye would be .09, .51, and 1.07%, respectively, an 11-fold difference between the high and low values. These data were calculated for the equation shown in Figure 7 but can also be calculated from the parameters given in Table 11 (data sets 9 to 11).

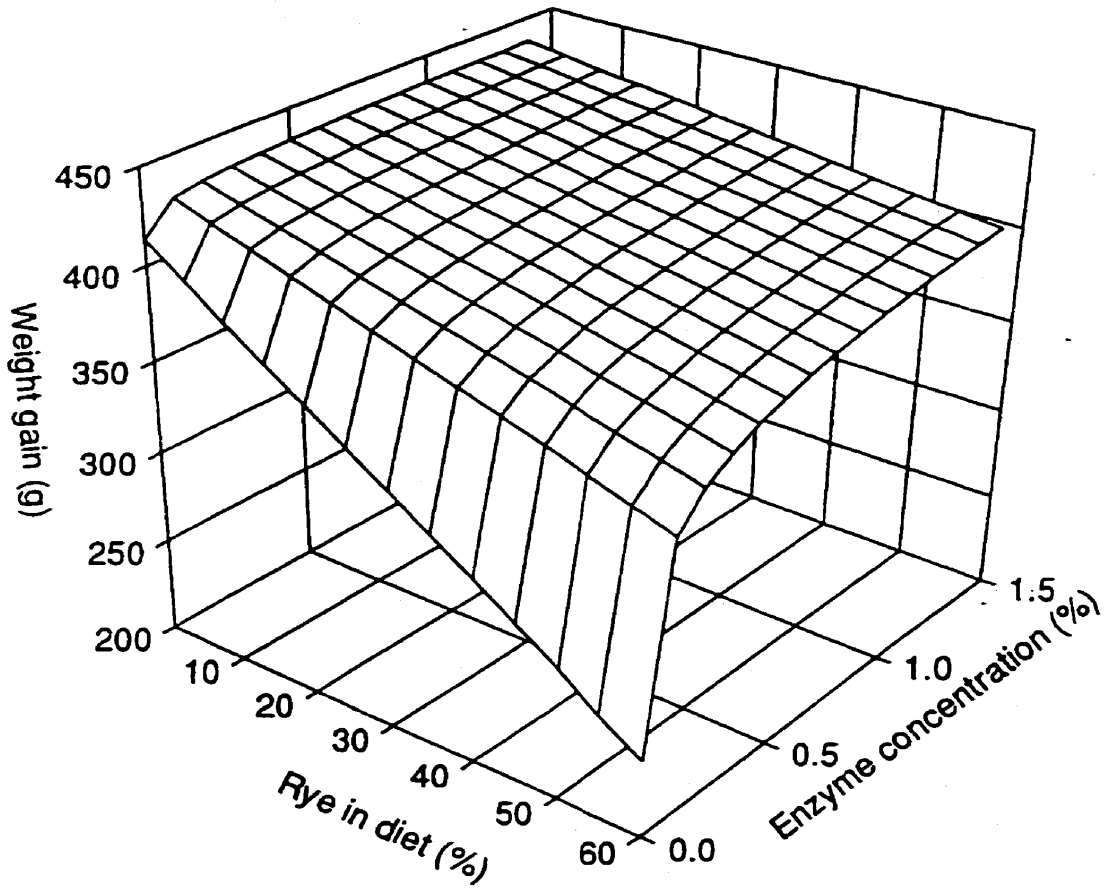

FIGURE 7. Effect of enzyme concentration (X) and rye content of diet (Z) on chick gain (Y). $Y = 436.11 + 7.58 \log X - .63 Z + .75 Z \log X$; all coefficients in the equation were significantly different from zero ($P < .001$) with the exception of the coefficient for log X ($P < .1$); $r^2 = .94$, residual SD = 12.56 g.

General Discussion

A similar model, as far as we are aware, has not been used before to predict the enhancement of the nutritive value of different diets by enzyme treatment. In this study, the model equation was able to predict the performance response of chicks when fed diets containing different amounts of an enzyme and different proportions of two cereals. In many of the comparisons, r^2 values of greater than .90 were obtained indicating that the prediction equation is accurate. The equation is also simple, because the improvement in performance with enzyme addition is directly related to the logarithmic concentration of the enzyme in the diet. The results demonstrate that for each ninefold increase in the amount of enzyme (i.e., an increase to 10 times the starting amount) there is a 100% improvement in chick performance. Under such conditions a small amount of enzyme may produce a significant improvement, whereas, near maximal improvement would require as much as 100- or 1000-times more enzyme. The data also suggests that much higher amounts of enzyme may be required for diets that contain a high amount rather than a low amount of the anti-nutritive factor. The efficacy of the enzyme can be readily obtained from the slope of the regression equation (B, performance change per log of units of enzyme in the diet) and is influenced by many factors, such as the amount of anti-nutritive factors in the diet (i.e., arbinoxylans), age and type of chick, type of enzyme used, method of expressing amount of enzyme added to the diet (i.e., units of activity vs. an amount per unit weight of diet), and the particular performance criterion measured (i.e., weight gain, feed to 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 regards to its effect on such other factors as nutrient digestion and absorption (unpublished

observations). Overall the model provides a new approach to establish efficacy of an enzyme when added to chicken diets and may possibly apply to other species of animals.

IMPLICATIONS

This study demonstrates that it is possible to accurately predict the response of chicks to dietary enzyme supplementation using a relatively simple equation. The equation shows that successive nine-fold increases in the amount of enzyme are required for each incremental improvement in animal performance; ie, if .1% enzyme produces a 10% increase in weight gain, then 1% enzyme (10 times more) would only produce an additional 10% improvement. The equation provides a simple means of estimating the efficacy of the enzyme and is influenced by factors such as type of enzyme, processing of the diet, type and variety of cereal fed, and class of poultry.

MANUSCRIPT 2

PREDICTION OF EFFECT OF ENZYMES ON CHICK PERFORMANCE WHEN ADDED TO CEREAL-BASED DIETS: USE OF A MODIFIED LOG-LINEAR MODEL

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ABSTRACT A previous study demonstrated that a log equation could be used to predict the relationship between the amount of a crude enzyme added to a diet and chick performance. The objective of the current study was to determine if a modification of the original equation, in conjunction with a computer program, would overcome some of its limitations. The modified equation was $Y = A + B \log (C X + 1)$, where Y is the estimated performance value; A is the intercept that represents the performance without enzyme supplementation; B, the slope of the equation (performance change per log unit of an enzyme in the diet), is a measure of an enzyme efficacy; C is an amplified factor; and X is the amount of enzyme in the diet. The results demonstrated that the new model more accurately predicted chick performance than that of the original equation with correlations (r) between chick performance and amount of different enzymes added to the diet ranging from $r = 0.80$ to 0.99 ($P < 0.05$). In addition, the same trends were found when the model was used to assess the efficacy of a given enzyme added to corn-, wheat-, barley-, and rye-based diets or for combinations of two dietary components (rye and wheat). The model proposed in this study provides a new means of assessing the overall efficacy of an enzyme preparation. This model could be routinely used by enzyme and livestock producers to establish the best combination of different cereals and enzymes so as to maximize net returns.

(*Key words*: prediction, efficacy, enzymes, chick performance, log-linear model)

INTRODUCTION

The use of enzymes in the animal feed industry has greatly expanded in the past 10 yr, especially in countries like Canada that utilize large quantities of cereals such as barley, oats, wheat and rye in poultry and pig diets. Enzymes are biological catalysts that are able to hydrolyze and thereby neutralize the negative effects produced by certain viscous compounds in these cereals. The compounds are referred to as nonstarch, water-soluble polysaccharides. Enzymes are able to eliminate the effects of the nonnutritive, nonstarch, water-soluble polysaccharides when added to poultry and pig diets, which results in increased efficacy of feed utilization, increased rates of growth, and reduced environmental pollution due to a decreased output of manure and gases such as ammonia (Campbell and Bedford, 1992; Chesson, 1993; Bedford, 1995; Marquardt, 1997; Marquardt and Bedford, 1997; Zhang et al., 1997).

Recently, we have developed a simple log-linear model using two sets of data and data from the literature to predict the response of chicks to dietary enzymes (Zhang et al., 1996). The model equation was able to predict the performance of chicks fed diets containing different amounts of enzyme and different proportions of two cereals. In many of the comparisons, r^2 values greater than 0.90 and in some studies as high as 0.99 were obtained, indicating that the prediction equation is highly accurate, especially where there is a significant response to enzyme treatment. The equation is also simple because the improvement in performance with enzyme addition is linearly related to the logarithmic concentration of enzyme in the diet. The overall efficacy of any enzyme preparation for a particular cereal or class of poultry with regard to any index of animal performance, such as weight gain and feed

to gain ratio, can be assessed from a single value B, the slope of the model equation (Marquardt et al., 1996; Zhang et al., 1996). In addition, we also compared this log-linear model with several other nonlinear models, such as a saturation model and a polynomial model (unpublished data). The results demonstrated that the models were either not a good fit (i.e., saturation model) or too complex to explain its parameters (i.e., polynomial model). Moreover, no other studies have demonstrated the ability of these models to accurately predict the response of chickens to a feed enzyme.

The model clearly demonstrates that there is a linear relationship between chick performance and the log of the amount of an enzyme added to the diet. This model can be used to1) predict chick performance for any amount of enzyme and any combination of cereals such as barley, oats, wheat, and rye when the values of enzyme activity or its relative concentrations are converted to logarithmic values, 2) assess the overall efficacy of an enzyme preparation when added to the diet, and 3) predict least-cost or economic return per unit of enzyme added to a diet when used in conjunction with other equations. However, the assigned ε value in the model equation, as defined in a previous study, $Y = A + B \log (X + \varepsilon)$, for the diet without enzyme addition can only be selected when input data are available (Zhang et al., 1996). This ε value is used to correct for log zero (no enzyme), which cannot be calculated. This correction results in two problems: 1) the correction does not provide accurate performance values, as indicated by the intercept of the model, A, when the diet contains no added enzyme (a negative control) and 2) an ε value or a set of ε values are difficult to assign to diets that not only contain different amounts of a given enzyme but also different proportions of two cereals (i.e., wheat and barley).

The purpose of this study was 1) to modify the previous model, 2) to demonstrate the utility of specific parameters that could be used to assess the efficacy of an enzyme when added to the diet (i.e., B, the slope of the equation), and 3) to calculate the parameters of the modified equation using the specially developed computer program. In addition, the implications of the modified model are discussed.

MATERIALS AND METHODS

Sources of Data

The data used in this study were obtained from three previous studies: Zhang et al. (1996), Bedford and Classen (1992), and Marquardt et al.(1994). In the first study, rye grain (Prima) was selected as the cereal in the diet because it contains high levels of viscous arabinoxylans (Antoniou et al., 1981). The soluble arabinoxylans in rye grains, which have been reported to have a concentration of 1.7 to 2.1% (Antoniou et al., 1981; Marquardt, 1997), are primarily responsible for their antinutritive effects (Antoniou et al., 1981). They greatly reduce chick performance but are efficiently hydrolyzed by enzyme preparations containing xylanase activity (Fengler et al., 1988; Fengler and Marquardt, 1988; Marquardt et al., 1994; Zhang et al., 1996, 1997). Two enzyme preparations, RM1 (a test sample)[1] and NQ,[2] were used in the first study (Zhang et al., 1996). The xylanase activity of the two enzyme preparations was assayed by the azo-dye method of McCleary (1992) using dye-labeled arabinoxylan as the substrate. These values were 389 and 778 U/g of enzyme preparation,

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[1]Finnfeeds International Ltd., Wiltshire, UK SN8 1XN.

[2]Nutri-Quest, Chesterfield, MO 63017.

respectively, and were the values used in the regression analysis study. The diet comprised the following ingredients: 60% rye, 8.25% wheat, 24.5% soybean meal, 2.4% vegetable oil, and 4.35% other ingredients. The final calculated protein concentration and MEn of the diets were 180 g/kg and 12.34 MJ/kg diet, respectively, which met the requirements for Leghorn chicks (NRC, 1994). In this experiment 1-d-old Single Comb White Leghorn cockerels were fed a commercial starter diet for a 7-d pre-experimental period, and after 4 h of food deprivation, were randomly distributed into experimental groups in such a way that all groups had the same average weight. The experimental diets were fed to birds from 7 to 21 d of age. The rye diets contained different concentrations of NQ and RM1 as shown in Table 12. Bird weight and feed consumption were recorded 4 h after removal of feed at 14 and 21 d of age (Zhang et al. 1996).

The data for the second study was obtained from Bedford and Classen (1992). In this experiment, 1-d-old male broiler chicks were fed four diets supplemented with different amounts of a pentosanase preparation (experimental product[1] from *Trichoderma longibrachiatum*) in a 4 x 6 factorial design from 1 to 19 d of age. The diets consisted of the following proportions of rye (Musketeer) and wheat (unknown): 0:60, 20:40, 40:20, and 60:0 with the other ingredients: 32.05% soybean meal, 4% corn oil, and 3.95% remaining ingredients. The calculated MEn of the four diets were 12.85, 12.50, 12.15, and 11.80 MJ/kg, respectively. The enzyme preparation added to each of the different diets was 0, 1, 2, 4, 8, and 16 g/kg. The xylanase activity of this enzyme preparation was 2,150 U, as determined by the reducing sugar method when assayed on oat spelt xylan (Seeta et al., 1989).

The data for the third study were obtained from the Experiment 2 of Marquardt et al.

TABLE 12. The relationship between the amount of enzymes (RM1 and NQ) added to a rye-based diet and chick performance (Experiment 1)[1]

		Enzyme preparation[2]										
Item[3]		RM1						NQ				
Enzyme												
Amount	%	0	0.25	0.75	2.25	6.75	20.3	0.1	0.3	0.9	2.7	8.1
	Log %	-	-0.6	-0.1	0.35	0.83	1.31	-1	-0.5	0	0.43	0.91
Activity	U/kg	0	97	292	875	2626	7877	79	233	700	2100	6302
	Log U/kg	-	1.99	2.47	2.94	3.42	3.9	1.89	2.37	2.85	3.32	3.8
WG[4] (g)												
Wk 1	Y_i	196	248	256	268	275	284	236	275	284	277	308
	Y_i-Y_0	0	52	60	72	79	88	40	79	88	81	112
Wk 2	Y_i	345	353	355	372	397	405	368	371	373	384	393
	Y_i-Y_0	0	8	10	27	52	60	13	16	18	29	38
2 wk	Y_i	541	601	611	640	672	690	604	646	657	661	701
	Y_i-Y_0	0	60	70	99	131	149	63	105	116	120	160
F/G[4] (g/g)												
Wk 1	Y_i	2.64	2.28	2.25	2.18	2.17	2.09	2.37	2.16	2.07	2.13	2.06
	Y_i-Y_0	0	-0.4	-0.4	-0.5	-0.47	-0.55	-0.3	-0.5	-0.6	-0.51	-0.58
Wk 2	Y_i	2.31	2.3	2.28	2.21	2.18	2.12	2.24	2.25	2.26	2.18	2.23
	Y_i-Y_0	0	0	0	-0.1	-0.13	-0.19	0	0	0	-0.13	-0.1
2 wk	Y_i	2.43	2.29	2.27	2.19	2.17	2.11	2.29	2.21	2.18	2.15	2.16
	Y_i-Y_0	0	-0.1	-0.2	-0.2	-0.26	-0.32	-0.1	-0.2	-0.3	-0.28	-0.27

[1]The raw data are from Zhang et al. (1996).

[2]Enzyme preparations, RM1 (Finnfeeds International Ltd., Wiltshire, UK SN8 1XN) and NQ (Nutri-Quest, Chesterfield, MO 63017), which contained 389 and 778 U/g of xylanase activity, were added to diets to give the indicated percentage or activity values (U/kg diet).

[3]Y_i and Y_0 represent the respective observed values of chick performance for the indicated periods when different concentrations of enzyme (i) or no enzyme (0) was added to the diet (0 to 7,877 U/kg for RM1 and 0 to 6,302 U/kg for NQ). These values are not the

calculated values. Y_i-Y_0 = net improvement in performance relative to the diet without enzyme addition when a certain concentration of enzyme is added to the diet.

[4]WG = weight gain; F/G = feed to gain ratio.

(1994). In this experiment, 1-d-old Single Comb White Leghorn chicks were fed a commercial starter diet for a 7-d pre-experimental period. The experimental diets were fed to birds from 7 to 21 d of age in a factorial arrangement of treatments: 4 (cereals) x 4 (enzyme doses). The cereals used in the diets were 63% corn (unknown), 67% wheat (Katepwa), 66% hulless barley (Scount), and 64% rye (Prima), respectively. The calculated MEn of the four diets were 3,024, 3,194, 2,976, and 2,869 kcal/kg. The diets were supplemented with different concentrations (0, 0.5, 1.0, and 2.0 g/kg) of a crude enzyme preparation (Kyowa Cellulase)[2]. The xylanase and cellulase activities as determined by the Japan Food Laboratory were 1,500 and 1,000 U/g, respectively.

Analyses of Data

Development of Computer Program. The outline of the program that was developed to calculate the parameters for the modified log-linear model was written in QuickBASIC[3] (Figure 8). The input for model derivation included data from chick performance such as weight gain (g) or feed to gain ratio (g/g) with the corresponding enzyme activity (U/kg diet) or amount of enzyme (%). A range for C was defined (usually 10^{-3} to 10^{10}), and the lowest value was the starting value. A least squares regression for each C values was evaluated between chick performance and the log of enzyme activity or log amount of enzyme (%). In order to obtain the best fit value, an iterative step was incorporated in which a set of C was calculated. The parameters selected for the equation (intercept A, slope B and constant C) were those that had the highest correlation coefficient (r^2) and the lowest standard error of the estimated mean (S_y), as determined by the method of least squares. The *F*-value was also

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[3]Microsoft Canada, Inc., 6300 Northwest Drive, Mississauga, Ontario, L4V 1J7, Canada.

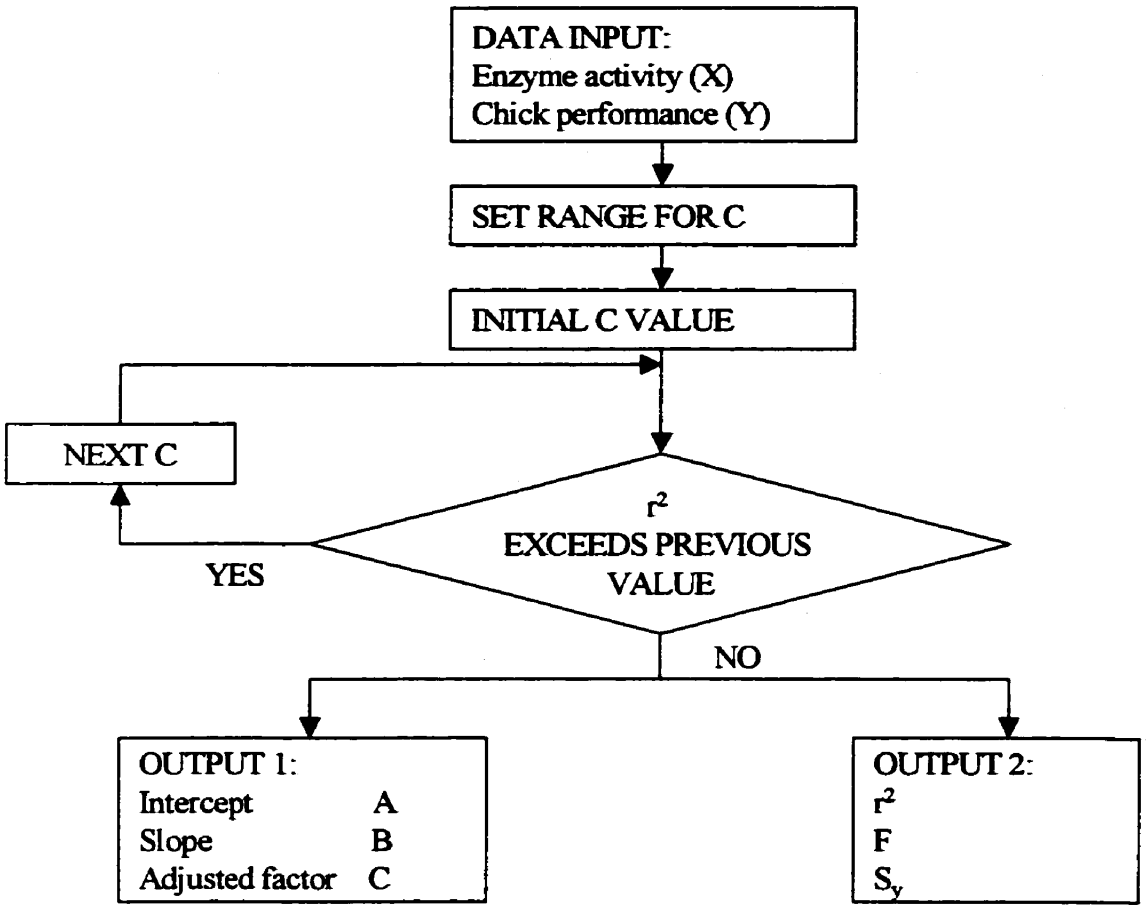

FIGURE 8. Flow diagram for BASIC program used to estimate the parameter of the prediction equation, $Y = A + B \log (C X + 1)$, where Y is the chick performance; X is the amount of enzyme in the diet; A is the intercept of the equation which represents the performance with enzyme supplementation; B, the slope of the equation, is a measure of enzyme efficacy; and C is an amplified factor. The correlation coefficient (r^2), the standard error of the estimated mean (S_y), and the *F*-value test for the best fit of the data set.

calculated to test the hypothesis of the regression model. The same r^2, Y, and A values were obtained, irrespective of whether the enzyme was expressed as a percentage or activity value. The value of the slope, however, was different, depending on whether it was expressed as performance per unit of activity or per relative amount of enzyme (%). These values can, nevertheless, be readily interconverted if activity and amounts of enzyme are known.

Statistical Analysis. Data for the feeding trials were analyzed according to the Statistical Analysis System (SAS, 1988). A completely randomized design was used with the experimental unit being a cage unit. Data from the previous experiments, as discussed above, were analyzed by ANOVA to determine significance of main effects. The means of the data were subjected to regression analysis to calculate the parameters of the modified equation $Y = A + B \log (C X + 1)$, where Y is the estimated performance value; A is the intercept that represents the performance without enzyme supplementation; B, the slope of the equation (performance change per log unit of an enzyme in the diet), is a measure of an enzyme efficacy; C is an amplified factor; and X is the amount of enzyme in the diet. The equation describes the relationship between the chick performance and the amount of enzyme added to the diet by using the computer program discussed above.

RESULTS AND DISCUSSION

Dose Response of an Enzyme When Added to Cereal-Based Diets

Dose-response studies are required to establish the nature of the response obtained per increment of enzyme when added to a specific type of diet. Such studies must be carried out 1) to establish the optimal amounts of xylanase and, possibly, arabinofuranosidase required

to degrade the target substrate, arabinoxylan, in wheat and rye or the amounts of xylanase, β-glucanase, and phytase that must be added to barley-, wheat-, and rye-based diets so that poultry and swine can efficiently utilize dietary nutrients; 2) to predict the least-cost analysis for the best return; and 3) to facilitate research on certain physiological functions such as the passage rate of digesta as affected by enzyme supplementation (Chesson, 1993; Forsberg et al., 1993; Guenter, 1997a, b; Marquardt and Bedford, 1997).

The results shown in Table 12 indicate that the amount of enzyme preparation in the rye-based diet had a marked effect on the performance of Single Comb White Leghorn chicks at different ages ($P < 0.05$). The overall improvements were 28 (149/541 x 100) and 13% for weight gain and 30 and 11% for feed to gain ratio during the 2-wk period for the RM1 and NQ enzyme preparations (Table 13). The responses in weight gain and feed to gain ratio for RM1 and NQ were greater during Week 1 [45, 22% and 57, 21%, respectively ($P < 0.05$)] than during Week 2 [17, 8%; and 14, 3% respectively ($P < 0.05$)]. In addition, our results demonstrated that the improvement in the performance of chicks due to an increasing amount of dietary enzyme was typical of that obtained with a mixed-order saturation curve (Lehninger et al., 1993; Zhang et al., 1996). The response in chick performance to enzyme addition was not only affected by the concentration of enzyme but by the age of the chicks and the type of enzyme used. The results of a previous study, however, clearly demonstrated that the response to enzyme under defined conditions was linearly related to the log of enzyme concentration but that there were some problems with this equation. This latter observation formed the basis for the development of the modified prediction model as outlined below. By using this equation, as shown subsequently, it is possible to accurately predict performance

TABLE 13. Evaluation of the performance of Single Com White Leghorn chicks fed a rye-based diet with different concentrations of enzymes (RM1 and NQ) using different parameters calculated in model Equations 1 and 2 (Experiment 1)[1, 2]

	RM1			NQ					
	(U/kg)			(U/kg)			(%)		
Item[3]	Wk 1	Wk 2	2 wk	Wk 1	Wk 2	2 wk	Wk 1	Wk 2	2 wk
WG[4] (g)									
A	195	334	529	191	342	534	194	344	538
(A_t)	187	329	515	184	334	519	278	378	656
Y_0	196	345	541	196	345	541	196	345	541
B	18	11	30	22	9.3	32	28	12	40
Y_m-Y_0	88	60	149	112	38	160	112	38	160
B/A×10^{-2}	9.2	3.4	5.6	12	2.7	5.9	14	3.4	7.3
(Y_m-Y_0)/Y_0	0.45	0.17	0.28	0.57	0.14	0.3	0.57	0.14	0.3
r^2	0.99	0.64	0.91	0.91	0.94	0.94	0.93	0.97	0.97
(r_t^2)	0.96	0.74	0.98	0.93	0.97	0.97	0.92	0.98	0.97
C	10	10	10	10	10	10	10^3	10^3	10^3
F/G[4] (g/g)									
A	2.64	2.35	2.45	2.65	2.31	2.43	2.65	2.31	2.43
(A_t)	2.67	2.37	2.48	2.67	2.31	2.45	2.15	2.23	2.2
Y_0	2.64	2.31	2.43	2.64	2.31	2.43	2.64	2.31	2.43
B×10^{-2}	-9.1	-3.4	-6.3	-12.7	-2	-6.1	-12.4	-2	-6
Y_m-Y_0	-0.55	-0.19	-0.3	-0.58	-0.1	-0.3	-0.58	-0.1	-0.27
B/A×10^{-2}	-3.4	-1.5	-2.6	-4.8	-0.9	-2.5	-4.7	-0.9	-2.5
(Y_m-Y_0)/Y_0	-0.21	-0.1	-0.1	-0.22	0	-0.1	-0.22	0	-0.11
r^2	0.99	0.62	0.94	0.92	0.66	0.96	0.92	0.66	0.95
(r_t^2)	0.93	0.76	0.99	0.89	0.62	0.93	0.91	0.65	0.95
C	10^2	10	10	10	10	10	10^4	10^4	10^4

[1] Data for this Table are from Zhang et al., 1996.

[2]RM1 enzyme preparation was from Finnfeed International Ltd., Wiltshire, UK SN8 1XN; NQ enzyme preparation was from Nutri-Quest, Chesterfield, MO 63017.

[3]A, B, and C are the parameters calculated from Equation 2, $Y = A + B \log (CX + 1)$, using the newly developed computer program, where Y is the chick performance [i.e., weight gain (g), feed to gain ratio (g/g)]; X is the enzyme preparation added to the diet (U/kg or %); A, the intercept of the equation, represents the chick performance without addition of an enzyme preparation (g, g/g); B, the slope of the equation, provides an index for evaluating the efficacy of an enzyme added to the diet (g or g/g per log U/kg; g or g/g per log %), and C is an adjusted factor used to correct the X value when enzyme is not added to the diet. X in Equations is the amount of enzyme (U/kg or %) added to the diet. A_1 values are the A values as estimated from Equation 1, $Y = A + B \log (X + \varepsilon)$ that were originally reported by Zhang et al. (1996). Y_m and Y_0 represent the respective observed performance values of chicks [weight gain (g) or feed to gain ratio (g/g)] for the indicated periods when the highest enzyme concentration or no enzyme was added to the different diets. r^2 is the correlation coefficient between the observed experimental values and predicted values when Equation 2 (r^2) or Equation 1 (r_1^2) was used.

[4]WG = weight gain; F/G = feed to gain ratio.

of chickens when fed different combinations of specific cereals and different concentrations of a given enzyme preparation.

Development of the Prediction Model

Zhang et al. (1996), based on the results of two experiments and published data, developed an accurate but simple model equation to predict the response of an animal to a feed enzyme. The criteria for developing the model were that there should be a good fit (high r^2, large F value and low standard deviation; SAS, 1988) between the observed and predicted data, that the model should be simple to interpret, and that it should provide information that is useful to animal scientists and enzymologists. The model equation was as follows:

$$Y = A + B \log X \qquad [0]$$

where Y is the estimated performance value [for example, weight gain (g)], X is the concentration of an enzyme (g or U/kg diet or % diet) added to the diet, and B is the slope of the equation (performance change per log unit of an enzyme in the diet). B is a measure of the efficacy of an enzyme preparation. A, the intercept (Y axis), theoretically represents performance without an enzyme added to the diet; however, this value is not readily obtained, as there is no value for the log of zero (the value without enzyme supplementation, i.e., when $X = 0$). Therefore Equation 0 cannot be used to calculate chick performance for diets that do not contain added enzyme. In order to obtain an A value, an amount of enzyme (ε) was selected that was very small and close to zero (Equation 1).

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

Zhang et al. (1996) reported that the ε value was constant, as it was not affected by enzyme concentration for a given diet. Therefore, it was possible to select an appropriate ε for

Equation 1 (high r^2 and low standard deviation).

The data from several experiments fitted this model (Equation 1) well ($r^2 > 0.90$; $P < 0.001$). The model appears to be universal in nature, as it can be used to describe the relationship among different enzyme concentrations; different enzyme preparations such as phytase; different classes of poultry and other livestock; and different amounts of a given cereal or different cereals. It may even be used to describe the effect of enzyme on the rate of absorption of nutrients (i.e., glucose or amino acid) in the gut of animals such as swine (Zhang et al., 1996; Marquardt, 1997).

Modification of the Log-linear Equation and the Application of Some of Its Parameters

Background. The model equation as described above not only can be used to describe the relationship between the amount of an enzyme added to a diet and its effect on chick performance, but can also be utilized for other applications. For example, the slope of the equation, B, is a measure of the overall efficacy of an enzyme and, therefore, provides a basis for comparison of all enzymes, each on the basis of a single value, the B value. However, the intercept, A, in Equation 1 actually represents chick performance when a preselected and substituted value for zero (ε) is used. As such $A = Y - B \log \varepsilon$ may not yield an accurate estimate of chick performance for diets that do not contain added enzyme. In turn, the selected ε may also affect the slope B of Equation 1 [$B = (Y-A) / \log (X + \varepsilon)$]. These are weaknesses of the previous model developed by Zhang et al. (1996). The following equations were utilized to overcome these weakness.

Modification of Equation 1. As discussed before, the introduction of an ε value into Equation 1 not only decreases the accuracy of certain parameters, such as A or B, but its

value is difficult to calculate; therefore, an arbitrary ε value must be selected (Zhang et al., 1996). A different approach as outlined below can be used to solve this problem. In this approach ε, in Equation 1, is assigned a value of 1 (ε = 1), and X is amplified several-fold by use of a constant (C). Therefore, the newly modified equation is as follows:

$$Y = A + B \log (C X + 1). \qquad [2]$$

The intent of this modification is the same as the ε treatment; that is, the value of 1 relative to CX should be very small as is ε relative to X. Equation 2, when X = 0 (i.e., without enzyme addition), therefore becomes:

$$Y = A + B \log (C \times 0 + 1). \qquad [3]$$

Because C × 0 = 0, and B log 1 = 0, then

$$Y_0 = A. \qquad [4]$$

The value of A in Equation 4 clearly indicates that it represents the predicted performance of chicks without addition of an enzyme preparation (Y_0), whereas this is not accurately estimated using the value of ε in Equation 1. Another problem is the calculation of the three parameters (A, B, and C) from Equation 2, as the equation is derived from only two variables (performance of the animal and the amount of enzyme). Based on the same criteria for selection of ε (Zhang et al., 1996), a computer program using BASIC language was developed based on a least squares procedure and a stepwise technique to calculate the different parameters (A, B, and C) of Equation 2 (Figure 8). In this program A, B and C values were selected when these values yielded a maximum r^2 and a minimum standard error (best fit values).

The results presented in Table 13 clearly demonstrate that in all cases the A value, as determined by use of Equation 2, provides a better measure of observed performance values (Y_0) than the A_i values that were derived using Equation 1. Similar results were obtained in Tables 14 and 15. These data indicate that Equation 2, in conjunction with the developed computer program, is more suitable than Equation 1, as it provides a more accurate estimate of the A value than Equation 1 as discussed above and overcomes the main shortcoming of Equation 1 (i.e., an arbitrary ε value used to calculate the log zero value).

The B Values

The Slope of the Equation and the Efficacy of an Enzyme. The B value, which is the slope of Equation 1 or 2, is a measure of performance per log unit of enzyme. This value can be considered a measure of the efficacy of any given enzyme preparation, irrespective of the amounts of enzyme used to establish this value. In one study (Table 12), similar activities of two enzyme preparations (RM1 and NQ) were added to the rye-based diet. The net improvement in performance ($Y_i - Y_0$) with different dietary concentrations of enzyme (i) was used to calculate the corresponding B values (Table 13). The respective B values of NQ compared to RM1 in the first week for weight gain and the feed to gain ratio were 22.3 vs. 18.0 g weight gain per log unit of enzyme added and −0.13 vs. −0.09 g gain/g feed intake per log unit of enzyme added. This result agrees with the trend observed for the corresponding overall net improvements ($Y_m - Y_0$) [i.e., 112 vs. 88 (g), and −0.58 vs. −0.55 (g/g)] during the same period (Table 13). In addition, the net performance of chick ($Y_m - Y_0$) on RM1 compared with NQ in the second week was 60 vs. 38 g for weight gain and −0.19 vs. −0.08 g/g for feed to gain ratio. The corresponding B values in Table 13 were 11.0 vs. 9.3 g per

TABLE 14. Evaluation of the performance of broiler chicks fed diets containing different proportions of rye and wheat and different amounts of enzyme as determined from different parameters calculated in Equation 1 or 2 (Experiment 2)[1]

	Rye[4] (%)							
Item[2,3]	0	20	40	60	0	20	40	60
	Enzyme, %				Enzyme, U/kg			
WG[5] (g)								
A	399	359	306	232	399	359	306	229
(A_1)	436	420	419	394	427	404	389	349
Y_0	398	359	306	233	398	359	306	233
B	3.6	6.28	21.6	54.4	2.48	6.84	20.2	49.3
Y_m-Y_0	31	61	110	166	31	61	110	166
B/A×10^{-2}	0.9	1.8	7	23.5	0.6	1.9	6.6	21.4
(Y_m-Y_0)/Y_0	0.078	0.17	0.359	0.712	0.078	0.17	0.359	0.712
r^2	0.65	0.99	0.96	0.96	0.67	0.99	0.96	0.95
(r_1^2)	0.49	0.92	0.94	0.96	0.67	0.99	0.96	0.95
C	10^{10}	10^{9}	10^{5}	10^{3}	10^{10}	10^{4}	10	10^{-2}
F/G[5] (g/g)								
A	1.63	1.73	1.83	2.28	1.63	1.73	1.83	2.28
(A_1)	1.5	1.56	1.64	1.62	1.53	1.6	1.69	1.8
Y_0	1.64	1.73	1.83	2.28	1.64	1.73	1.83	2.28
B×10^{-2}	-1.36	-1.62	-1.82	-8.83	-0.9	-1.1	-1.4	-9.8
Y_m-Y_0	-0.08	-0.11	-0.19	-0.62	-0.08	-0.11	-0.19	-0.62
B/A×10^{-3}	-8.35	-9.32	-9.92	-38.7	-5.52	-6.35	-7.65	-43.0
(Y_m-Y_0)/Y_0	-0.049	-0.064	-0.104	-0.272	-0.049	-0.064	-0.104	-0.272
r^2	0.65	0.72	0.7	0.99	0.68	0.71	0.7	0.99
(r_1^2)	0.43	0.65	0.62	0.95	0.49	0.71	0.67	0.98
C	10^{10}	10^{10}	10^{10}	10^{7}	10^{10}	10^{10}	10^{8}	10^{2}

[1] Data were calculated from that reported by Bedford and Classen (1992).

[2] A, B and C are the parameters calculated from Equation 2 using the developed computer program. The relative units for A and B are grams or grams per gram (A) and

grams or grams per gram per log units per kilogram or grams per gram per log percentage of enzyme added to the diet (B), for weight gain or feed to gain ratio, respectively. Parameter C is the amplified factor. The A_L values, in contrast to A, were calculated with Equation 1. r^2 and r_L^2 values are the correlation coefficients between the observed experimental values and the predicted values when Equation 1 (r_L^2) or Equation 2 (r^2) was used.

[3]Y_m and Y_0 represent the observed performance values of chicks [weight gain (g) or feed to gain ratio (g/g)] when either the highest enzyme concentration or no enzyme was added to the different diet.

[4]The values refer to percentage rye gain in diet. Wheat was the other cereal. Total cereal in diet was 60%.

[5]WG = weight gain; F/G = feed to gain ratio.

Table 15. Evaluation of the performance of Single Comb White Leghorn chicks fed different cereal-based diets with different concentrations of enzymes as determined from parameters calculated in Equation 1 or 2 (Experiment 3)[1]

Item[2,3]	Corn	Wheat	Barley	Rye	Corn	Wheat	Barley	Rye
	Enzyme, %				Enzyme, U/kg			
WG[4] (g)								
A	135	125	113	97	135	125	113	97
(A_t)	130	132	133	123	136	124	110	92
Y_0	135	125	113	97	135	125	113	97
B	-1.67	0.81	2.26	8.76	-1.2	0.55	1.67	6.33
Y_m-Y_0	-6	7	22	29	-6	7	22	29
B/A×10^{-2}	-1.2	0.6	2	9	-0.9	0.4	1.5	6.5
(Y_{in}-Y_0)/Y_0	-0.044	0.056	0.195	0.299	-0.04	0.056	0.195	0.3
r^2	0.92	0.96	0.98	0.99	0.92	0.97	0.97	0.99
(r_t^2)	0.93	0.92	0.96	0.99	0.93	0.91	0.95	0.99
C	10^{4}	10	10^{10}	10^{4}	10	10^{10}	10^{9}	10
F/G[4] (g/g)								
A	2.04	1.99	1.95	2.28	2.04	1.99	1.95	2.28
(A_t)	2.04	2.01	1.97	2.33	2.04	2.01	1.97	2.33
Y_0	2.04	1.99	1.95	2.28	2.04	1.99	1.95	2.28
B×10^{-2}	3.9	-2.8	-1.3	-6.8	3.5	-2	-0.9	-6.5
Y_m-Y_0	0.02	-0.1	-0.13	-0.29	0.02	-0.1	-0.13	-0.3
B/A×10^{-2}	1.9	-1.4	-0.7	-3	1.7	-1	-0.5	-2
(Y_m-Y_0)/Y_0	0.01	-0.05	-0.07	-0.13	0.01	-0.05	-0.1	-0.1
r^2	0.96	0.99	0.9	0.99	0.78	0.98	0.9	0.99
(r_t^2)	0.89	0.99	0.88	0.99	0.8	0.99	0.88	0.99
C	10	10^{4}	10^{10}	10^{5}	10	10	10^{10}	10

[1]The data are from Marquardt et al.(1994).

[2]A, B, and C are the parameters calculated from Equation 2 by using the newly developed computer program. The relative units for A and B are grams or grams per gram (A) and grams or grams per gram per log units per kilogram or grams per gram per log

percentage of enzyme added to the diet (B), for weight gain or feed to gain ratio, respectively. The parameter C is the adjusted factor. A_1 and A values were calculated using Equation 1 or Equation 2, respectively.

[3]Y_m and Y_0 represent the observed performance values of chicks [weight gain (g) or feed to gain ratio (g/g)] when either the highest enzyme concentration or no enzyme was added to the different diets.

[4]WG = weight gain; F/G = feed to gain ratio.

log unit of enzyme added for weight gain and −0.034 vs. −0.020 g/g per log unit of enzyme added for feed to gain ratio. Although the B values and the $Y_m - Y_0$ values followed a similar trend (r = 0.99, $P < 0.001$), they did not change proportionately.

These relationships are also supported by analysis of other data from the literature (Table 14). Bedford and Classen (1992) carried out a dose-response experiment of an enzyme preparation by using four different ratios of two cereals (i.e., wheat to rye, 0:60, 20:40, 40:20, 60:0) and six doses of enzyme (0, 0.1, 0.2, 0.4, 0.8, and 1.6%). Their results demonstrated that the net performance response of chicks to an enzyme preparation that was high in xylanase activity increased dramatically as the proportion of rye was increased. The relevant B values for weight gain during the 2-wk experimental period for diets containing 60, 40, 20 and0 % rye as calculated by Equation 2 were 54.4, 26.1, 6.3, and 0.36. The B values for feed to gain ratio were −0.103, −0.018, −0.016, and −0.013, respectively. The B values, as were the improvements in observed performance values ($Y_m - Y_0$), were considerably higher for diets containing a high, compared to a low, concentration of rye, which also reflects the overall efficacies of an enzyme to degrade the different concentrations of its antinutritional substrate (i.e., arabinoxylan) in the diet. Again as shown in Table 14, the B values and $Y_m - Y_0$ were highly related (r = 0.95, $P < 0.001$), but they did not change proportionally with the percentage of rye in the diet.

Another dose-response study was conducted by Marquardt et al. (1994), who studied the effect of different concentrations of an enzyme (high in both xylanase and β-glucanase activity) when added to diets containing high concentrations of different cereals and corn (i.e., corn, 63%; wheat, 67%; barley, 66%; and rye, 64%) on the performance of Leghorn chicks.

Their results indicated similar trends for the effect of the enzyme on chick performance and the B value as calculated from Equation 2. For example, the observed weight gain values ($Y_m - Y_0$) in Table 15 for the corn, wheat, barley, and rye diets were −6, 7, 22, and 29 g, respectively, whereas the B values calculated from Equation 2 were −1.67, 0.0.81, 2.26, and 8.76 g/log of percentage enzyme added to the diets, respectively. The correlation between the two sets of data were r = 0.90 ($P < 0.001$). Therefore, the B value is a measure of performance change per log of enzyme concentration and shows a linear relationship between performance and amount of enzyme in the diet, whereas the ($Y_m - Y_0$)/amount of enzyme yields a curvilinear relationship for net improvements per unit of enzyme. As a result, the former value is constant for any given set of data, irrespective of the amount of enzyme used, whereas the ($Y_m - Y_0$)/amount of enzyme under the same conditions decrease with increasing amounts of enzyme added to the diet. These data demonstrate that the B value provides a value that can be used to assess overall efficacy of a given enzyme, whereas the ($Y_m - Y_0$)/amount of enzyme value cannot provide such a value. Also, the B value could be estimated from as few as two data points, whereas this cannot be achieved from ($Y_m - Y_0$)/amount of enzyme.

Therefore, the slope of Equation 2 yields a single value, the B value, which is an index of the efficacy of an enzyme. This value not only measures the efficacy of different enzymes when the same diet is used but can also provide a measure of the efficacy of an enzyme when added to different diets or when different amounts of their target substrates are present in the diet. Practically speaking, the B value represents the net improvement of performance per log unit of an enzyme added to the diet as shown in the following Equation 5:

$$B = (Y - A) / \log (C X + 1). \quad [5]$$

Relative B value (B/A) and the Relative Efficacy of an Enzyme. The relative improvement of chick performance [$(Y_i - Y_0)/Y_0$ or $(Y_m - Y_0) / Y_0$] for RM1 and NQ is greater in the first week than in the second week with the value for the two periods being approximately the mean of the two values between them (Table 12 and 13). In contrast, the B values in Table 13 are not approximate and are additive for Weeks 1 and 2, which are consistent with the equation. However, a more meaningful comparison among B values would be weight gain per log unit of enzyme divided by the weight gain without enzyme, which would eliminate weight differences caused by different treatment times. The form of Equation 5 can, therefore, be changed as follows:

$$B / A = [(Y - A) / A] / \log (C X + 1). \quad [6]$$

The right side of Equation 6 represents the relative improvement in chick performance per log unit of an enzyme, and the B/A value is a B value relative to A. It can be simply called the relative B value. This value, (B/A), which could be considered as the relative efficacy of an enzyme, is given in Tables 13, 14, and 15.

The B Values and the Overall Efficacy of a Dietary Enzyme. From Equation 2, we know that the efficacy of an enzyme preparation, B, is a constant value, independent of the activity of the enzyme added to a given diet. However, the B values calculated from the data of Bedford and Classen (1992) and Marquardt et al. (1994) indicated that the efficacy of an enzyme will change with the concentration of the viscous substrates in the diet such as arabinoxylans. It is not clear, however, why the efficacy of an enzyme preparation varies with the concentration of substrates rather than being constant as expected. The reason for this

result may be that the efficacy of an enzyme relative to its substrate is constant, irrespective of the amount of antinutritive factor present in the diet. The overall efficacy of an enzyme preparation, as measured by the degree of improvement in chick performance, is variable and is a function of amount and type of dietary enzyme and the amount of antinutritive factors in the diet. Diets containing high levels of rye, wheat (in some studies), barley, or oats can cause severe problems for growing chicks that have been attributed to the highly viscous nonstarch polysaccharides. The lower nutritive value of these cereals is probably related to their high content of water-soluble arabinoxylans in rye and wheat and β-glucans in barley and oats. These highly viscous compounds decrease digestibility and absorption of all nutrients as they prevent access of digestive enzymes to the nutrients and the movement of the nutrients in the intestinal lumen (Antoniou et al., 1981; Fengler et al., 1988; Annison and Choct, 1991; Bedford, 1997b). The addition of enzyme preparations containing xylanase and β-glucanase activity overcomes the antinutritive problems of these cereals, as their viscosities can be reduced to values similar to that of water. Under such conditions, enzyme addition can dramatically improve chick performance and yield a correspondingly high B value. In contrast, if the cereals have low concentrations of the viscous carbohydrates, enzyme additions would have little effect on chick performance, and the enzyme would not be considered efficacious and would yield a low B value. Therefore, the overall efficacy of an enzyme on chick performance depends upon the amount and type of enzyme, and the amount and type of its target substrates in the diet, the maturity of the digestive system, and other factors.

Other Applications of the B Value. To our knowledge, there is no suitable standard to evaluate the quality of an enzyme preparation as an additive in animal feeds (Marquardt,

1997). It is difficult to make a meaningful comparison among different enzyme preparations based on stated activity values, as conditions vary for the assay of a given enzyme such as pH, temperature, and substrates used. In addition, the efficacy of an enzyme preparation is sometimes not simply dependent on its activity as determined by in vitro assay. The efficacy may be influenced by the relationship between its pH optimum and the pH at the site where hydrolysis occurs in the gut, its ability to resist the low pH and proteolytic activity in the digestion tract, and the presence of other enzymes that may synergistically enhance or antagonize its activity. Feeding studies are commonly used to evaluate the quality of different enzyme preparations. Usually, 0.1 to 0.3% (log value = −1 to −0.52) enzyme is added to the test diet, according to recommendations of most companies. However, it is difficult or impossible to determine the true efficacy of enzyme by using enzyme assays or dose-response feeding trials. This situation is applicable to previous dose-response studies, especially those in which relatively small differences in amounts of enzyme were used. The use of the B and B/A values from the Equations 2 and 5 should, therefore, provide the nutritionist with a useful and new approach to evaluate the efficacy of an enzyme preparation when added to the diet. In addition, it may be possible to use these values to: 1) determine the site of action for an enzyme preparation in the gut, 2) design a new enzyme preparation having a high efficacy (i.e., high B value instead of high enzyme activity under arbitrary conditions), 3) develop an in vitro method to evaluate the quality of an enzyme preparation, and 4) study the relationship between the B or B/A value and the turnover number, Kcat, Kcat / Km, etc., for an enzyme. The focus of basic research by scientists could attempt to more clearly resolve some of the many problems associated with the application of enzyme research in animals.

The C Value

Value C in Equation 2 is the adjusted factor calculated from the computer program and is required to calculate performance of chicks fed the diet without added enzyme. The values of C for NQ using enzyme activity and expressed as units per kilogram and percentage of the diet were 10 and 10^3 for weight gain or 10^4 for feed to gain ratio as shown in Table 13. In addition, the ratio between the two terms expressed (CX and 1) as units of NQ was approximately 10^3. Therefore, the addition of 1 to the CX number is relatively insignificant. Similar large ratios were also used in Tables 14 and 15. These values for C guarantee that 1, relative to CX, is very small, and as a result the actual and the calculated values for chick performance without added enzyme are essentially the same (see Tables 13 to 15). The use of the adjusted factor C in Equation 2 only resulted in a parallel movement of the regression line with a constant unit (B log C) when 1 relative to CX was very small. Therefore, it theoretically will not affect the calculated overall response when different amounts of enzyme (X) are added to the diet. Further research needs to be carried out to indicate whether the value of C might provide other useful information such as an index of experiment variation. Also this model is suitable only as an analytical model of experimental data and currently cannot be used as a general prediction model, as C changes depending upon the conditions under which the birds are reared.

Other Factors Affecting the Prediction Equation

Throughout the study, the amount of enzyme was expressed as either units per kilogram of feed or a percentage of the feed. Either value can be used to accurately predict chick performance as the r^2 values, when either is used, are in all cases nearly the same (Tables 13,

14, and15). For example, in Table 13 the r^2 values for feed to gain ratio with NQ enzyme for Wk 1, Wk 2 and 2 wk were 0.92, 0.66, and 0.96, irrespective of whether units per kilogram or percentage enzyme values were used in the equation. Likewise, the corresponding $Y_m - Y_0$ values were almost the same. The B and C values, however, are different as they depend on the whether values of are expressed as units or as an amount. Therefore, within a given data set, the ability to predict chick performance is independent of how the amount of enzyme is expressed. This statement would also apply to studies in which the antinutritive, viscous carbohydrates were the same and if the enzymes were the same. However, an accurate prediction of chick performance to feed enzymes by use of the equation is affected by many factors including age of chickens, activity of enzyme at the site at which digestion occurs, the absolute amount of enzyme in a given preparation or its activity as determined under specified conditions of temperature, substrate type and concentration, and pH. Currently, no standard reference target substrate is available for the different enzymes. The sites of action of enzymes have not been identified, and standard enzyme assays have not be developed. These problems have been discussed in greater detail by Marquardt and Bedford (1997) and Zhang et al. (2000a). Standardization of enzyme assays would, therefore, further improve the accuracy of the prediction equation when different enzyme preparations are used.

Least-cost Analysis with the Equation

Equation 2 indicates that the response to an enzyme supplementation is a function of enzyme concentration when converted to a logarithmic value for any given feedstuffs. It can also simultaneously predict the response to any proportion of two cereal diets supplemented with any given amount of an enzyme preparation (Zhang et al., 1996). Therefore, it is possible

to correctly estimate the least-cost economic return per unit of an enzyme added to a diet, provided accurate input data are available. This estimate would provide a means of determining the optimal amount of different cereals and supplemental enzyme that should be added to a diet to maximize economic return. By using this analysis, the prices that can be paid for an enzyme preparation or substitute cereals could be estimated for a maximum net return. A simple computer program with several applications included in predicting the price of an enzyme, the optimum amount of enzyme addition when maximum economic return is required, and the price and amount of a substituted cereal could be developed.

In conclusion, this study has demonstrated that it is possible to accurately estimate the overall efficacy of an enzyme preparation when added to the diet of poultry by using a new model equation. Future studies using this model will further demonstrate its utility for basic and applied research and as a tool in assessing economic return when feed enzymes are used.

MANUSCRIPT 3

PREDICTING AND EVALUATING THE PROFITABLE EFFECT OF FEED ENZYMES: USE OF A LOG-LINEAR MODEL IN POULTRY

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ABSTRACT Previous studies demonstrated that a log-linear equation could accurately predict chick performance when a feed enzyme was added to a diet and that the slope of the equation provided a measure of the efficacy of different enzymes. The objective of the study was to develop a software package from the equation, a Multiple Purpose Enzyme Analyzer (MPEA), for evaluating the profitable effect of enzymes when added to poultry feeds. A high correlation between the efficacy of different feed enzymes (B values, the slopes of the equations) and the maximal profits was obtained when feed enzymes were added to a barley-based diet ($r^2 = 0.99$, $P < 0.005$). In contrast, there was a low correlation between the B values and the maximal profits when a feed enzyme was added to different cereal-based diets ($r^2 = 0.61$, $P = 0.2171$). This suggested that there is not always a close association between efficacy of an enzyme when added to different cereals and the corresponding profitability. The MPEA was highly versatile as any combination of inputs such as the amounts of a feed enzyme and a substituted cereal required to yield a profit level could be determined. In conclusion, the MPEA can accurately evaluate profitability of using different feed enzymes, select the most profitable cereal for a given feed enzyme, determine the optimal amounts of a feed enzyme and/or a cereal, and even estimate the alternate price for a feed enzyme and a cereal. It should provide a useful tool for nutritionists.

(*Key words*: feed enzymes, maximal profit, profit functions, log-linear model)

INTRODUCTION

There has been increasing interest in quantitatively studying the effect of different levels of feed enzymes (inputs) when added to a diet on the performance (outputs) of chickens (Friesen et al., 1991; Bedford and Classen, 1992; Marquardt et al., 1994; and Zhang et al., 1996; 2000 b). The primary objectives of the former studies were 1) to estimate the optimal level of feed enzyme addition required to obtain maximal chick performance (Friesen et al., 1991; Bedford and Classen, 1992), and 2) to evaluate the efficacy of feed enzymes added to a diet (Rotter et al., 1989b; Zhang et al., 1996; 2000a, b). Frequently, the experimental designs and statistical procedures have only provided trends on the effects of enzyme treatment but have not provided precise prediction values that can be obtained when a given enzyme is added to a given diet. Therefore, it has been impossible to accurately estimate the relationship between inputs (enzyme or cereal) on outputs (chick performance), or to establish the most profitable combination of inputs for a specified output. In addition, researchers in nutrition have generally been concerned only with biological rather than economic criteria to evaluate and make recommendations on the effects of feed enzymes. The criteria that have been used for the evaluation of performance often were the treatments yielding the largest weight gain or the lowest feed to gain ratio per unit of enzyme addition (Friesen et al., 1991; Bedford and Classen, 1992). However, these maxima or minima have seldom been utilized to estimate the most profitable output or optimal input. Even where the objective was the prediction of the physical maxima or minima, the exact values could only be accurately estimated by use of a prediction equation (Zhang et al., 1996).

Recently, we have developed a simple log-linear model to accurately predict the response of chickens to dietary enzymes (Zhang et al., 1996). The model equation was able to predict the performance of chickens fed diets containing different amounts of an enzyme and different proportions of two cereals. Simple but accurate log-linear equations were derived from many previous dose response studies with feed enzymes, even though they were not designed for this purpose. In addition, the efficacy of any enzyme preparation for a particular cereal or class of poultry with regards to any index of animal performance such as weight gain or feed to gain ratio could be assayed from a single value of B, the slope of the model equation (Zhang et al., 1996, 2000b; Marquardt and Bedford, 1997). Therefore, it should be possible, using this equation along with other analyses, to correctly estimate the maximum economic return obtained when a feed enzyme is added to a diet. A requirement is that accurate input data be available. The objective of this study was to develop a Multiple Purpose Enzyme Analyzer (MPEA) for estimating the profitability of using enzymes in poultry feeds. Three main applications of the MPEA were to: 1) evaluate the effects of different enzyme preparations when they are added to a cereal-based diet using maximal profit as a standard, 2) determine the amounts of an enzyme preparation and/or a substituted cereal that should be used in a diet to obtain maximal profit, and 3) to establish the relationships among the price of an enzyme preparation, the price of the substituted cereal, and the economic return. Therefore, the use of the modelling method in conjunction with nutrition knowledge and computer technology should provide researchers and managers that use feed enzymes a powerful new approach for the analysis and interpretation of their data.

MATERIALS AND METHODS

Sources of Data

The data used in this study were obtained from four previous studies: Rotter et al. (1989b), Zhang et al. (1996), Bedford and Classen (1992), Marquardt et al. (1994). The data for the first study was obtained from Rotter et al. (1989b) and Zhang et al. (1996). In Experiment 3 of the study from Rotter et al. (1989b), 1-d-old Single Comb White Leghorn chicks were fed a commercial starter crumble for a 7-d pre-experimental period. A barley-based diet was fed to birds from 7 to 14 d of age in a completely randomized design. The diet consisted of the following ingredients: 65.50% barley, 23.07% soybean meal, and 11.43% other ingredients. The calculated MEn of the diet was 12.20 MJ/kg. Five enzyme preparations used in the study were: Cellulase Tv concentrate (*Trichoderma viride*)[1], Celluclast (*T. ressei*)[2], SP249 (*Aspergillus niger*)[2], Finizym (*A. niger*)[2], and Cereflo (*Bacillus subtilis*)[2]. The enzyme activities as determined by manufacturer were: Cellulast, 1633 NCU/g; SP249, 11240 PGU/g; Finizym, 217 FBG/g; Cellulase Tv concentrate 23880 CU/g; and Cereflo, 67.5 KNU/g. All of the enzymes at amounts of 0.003125, 0.00625, and 0.0125 % were added to the experimental diet for the dose-response study. Performances per chick were recorded at 14 d of age.

Another data set used in the first study was obtained from Zhang et al. (1996). In this experiment, 1-d-old Single Comb White Leghorn cockerels were fed a commercial starter diet for a 7-d pre-experimental period. The experimental diets were fed to birds from 7 to 21 d of

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[1]Miles Laboratories Inc., Elkhart, IN.

[2]Novo A/S, Bagsvaerd, Denmark.

age. The diet consisted of the following ingredients: 60% rye, 8.25% wheat, 24.5% soybean meal, and 6.75% other ingredients. The calculated MEn of the diet was 12.34 MJ/kg. Rye grain (Prima) was selected as the substituted cereal for wheat in the diet as it contains high levels of viscous arabinoxylans. The arabinoxylans in rye grains are primarily responsible for its antinutritive effects (Antoniou et al., 1981). They greatly reduce chick performance but are efficiently hydrolysed by enzyme preparations containing xylanase activity (Fengler et al., 1988; Fengler and Marquardt, 1988; Marquardt et al., 1994; Zhang et al., 1996, 1997). Two enzyme preparations, RM1 (*T. longibrachiatum*)[3] and NQ (*T. reesei*),[4] were used in this study (Zhang et al., 1996). The xylanase activity of RM1 and NQ was 389 and 778 U/g of enzyme preparation as assayed by the azo-dye method (McCleary, 1992) using dye-labelled arabinoxylan as the substrate. Different amounts of RM1 (0, 0.25, 0.75, 2.75, 6.75, 20.25 g/kg) and NQ (0, 0.1, 0.3, 0.9, 2.7, and 8.1 g/kg) were added to the diet, at the expense of rye, for a total 12 different treatments. Bird weight and feed consumption based on six birds were recorded 4 h after removal of feed at 21 d of age.

The data for the second study were obtained from experiment 2, Marquardt et al. (1994). In this experiment, 1-d-old Single Comb White Leghorn chicks were fed a commercial starter diet for a 7-d pre-experimental period. The experimental diets were fed to birds from 7 to 21 d of age in a factorial arrangement of treatments: 4 (cereals) x 4 (enzyme doses). The cereals used in four diets were 63% corn (unknown variety), 67% wheat (Katepwa), 66% hulless barley (Scout), and 64 % rye (Prima), respectively. The calculated MEn of the four diets were

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[3]Finnfeeds International Ltd., Wiltshire, UK SN8 1XN.

[4]Nutri-Quest, Chesterfield, MO, 63017.

12.66, 13.37, 12.46, and 12.01 MJ/kg. The diets were supplemented with different concentrations (0, 0.5, 1, and 2 g/kg) of a crude enzyme preparation, Kyowa Cellulase (*T. reesei*)[5]. The xylanase and cellulase activities as determined by the Japan Food Laboratory were 1500 and 1000 U/g, respectively. The chick performances based on a bird basis were recorded at 21 d of age.

The data for the third study was obtained from Bedford and Classen (1992). In this experiment,

1-d-old male broiler chicks were fed four diets supplemented with different amounts of a pentosanase preparation (experimental product[6] from *T. longibrachiatum*) in a 4 x 6 factorial arrangement of treatments from 1 to 19 d of age. The diets consisted of the following proportions of rye (Musketeer) and wheat (unknown variety): 0:60, 20:40, 40:20, and 60:0 each with 32.05% soybean meal, and 7.95% other ingredients. The calculated MEn of the four diets were 12.85, 12.50, 12.15, and 11.80 MJ/kg, respectively. The enzyme preparation added to each of the four diets was 0, 1, 2, 4, 8, and 16 g/kg. The xylanase activity of this enzyme preparation was 2,150 U/g as determined by the reducing sugar method when assayed on oat spelt xylan (Seeta et al., 1989). Chick performances, based on six birds, were recorded at 19 d of age.

For demonstration purposes, the price range for enzyme preparations was assumed to be from $3 to $7 per kg. The price for corn, wheat, barley, and rye was assumed to be $0.13, $0.12, $0.08, $0.08 per kg, respectively. The barley and rye were used as substituted cereals for wheat in the diet, therefore, their price was assumed to be less expensive than that of wheat. The average price of other ingredients in a diet was $0.08 per kg and the price of

chickens was $1.23 per kg live weight. Other prices can be inserted into the equations as desired as outlined below.

Outline of a MPEA

We have developed a log-linear model equation to predict the performance of chickens fed a cereal-based diet supplemented with different concentrations of a feed enzyme. The model can estimate maximal economic return when a feed enzyme is added to a diet. Based on the log-linear model, we have further developed a software package, a MPEA. The MPEA consists of two parts: a modelling and application part (Figure 9). The modelling part has revenue, production cost, and profit functions. The applications of MPEA are at least three. The first application is the evaluation of the profitable efficacy of different enzyme preparations when they are added to a specific diet and the determination of the most profitable cereal for a specific enzyme preparation based on maximal economic returns. The second is to determine the optimal amount of a feed enzyme and a cereal used in a diet to obtain maximal profit. The third is to determine the alternate price that should be paid for a given enzyme preparation and a cereal.

Principle of the MPEA: Maximal Profit with the Optimal Inputs

From the dose response study with varying the levels of a feed enzyme added to a diet, the log-linear model equation was selected to fit the data of the output or chick performance and the input or amount of enzyme. The general models as proposed by Zhang et al. (1996) for weight gain (Equation 1) and feed intake (Equation 2) were:

$$Y = A + B \log (C X_E + 1) \quad [1]$$

$$F = a + b \log (c X_E + 1) \quad [2]$$

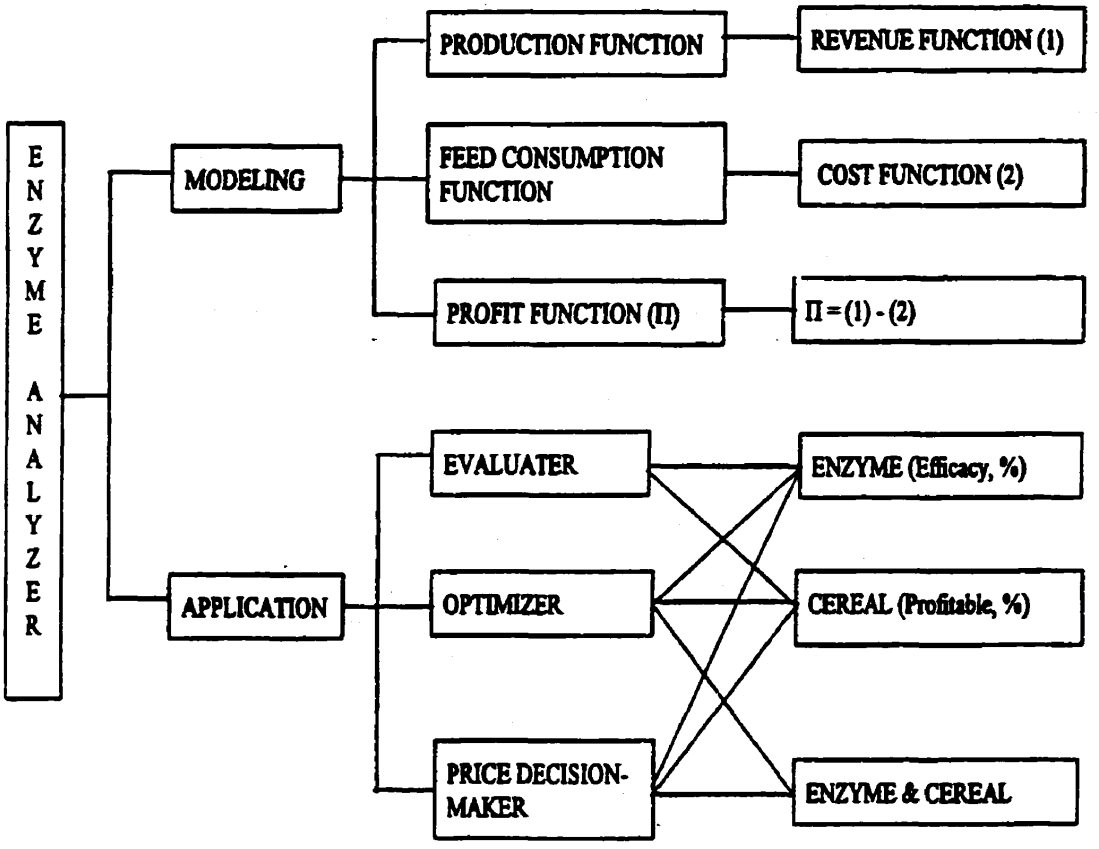

FIGURE 9. Principle and application of the Multiple Purpose Enzyme Analyzer for use of enzymes in poultry feeds.

where, X_E was the amount of an enzyme (percentage of diet), Y and F were weight gain (g) and feed intake (g), A, B, C and a, b, c were the corresponding coefficients of the two regression equations, respectively. Based on the two equations, we have developed a profit function (Equation 3) in general:

$$\Pi = P_y Y - F \sum (P_i X_i) \qquad [3]$$

where, Π is the profit, P_y is the price of chickens, P_i is the price of ingredients in a diet and X_i is the amount (%) of the i-th variable such as rye, wheat, enzyme preparation, and other ingredients in a diet. The maximal profit can, therefore, be calculated when the partial derivatives of Equation 3 are equal to zero (Heady and Dillon, 1961). A more detailed profit equation can be deduced when other variables are utilized (Equation 4). Let us use the data set from Bedford and Classen (1992) as an example, the optimal amounts of a feed enzyme and a substituted cereal (rye) for wheat in a diet for maximum profit can be determined using the following objective function:

$$\Pi = P_y Y - F (P_W X_W + P_R X_R + P_E X_E + P_O X_O) \qquad [4]$$

where: Y (weight gain, kg) and F (feed intake, kg) are the regression equations 1 and 2 for estimating the revenue function (P_y Y) and the cost function {F ($P_W X_W + P_R X_R + P_E X_E + P_O X_O$)} in Equation 4. We let P_y (\$ / kg) = price of chicken per kg, P_w = price of wheat per kg, P_R = price of rye per kg, P_E = price of enzyme per kg, and P_O = price of other ingredients per kg; and assume wheat is a standard cereal used in a diet, and rye, a cheaper cereal relative to wheat, is selected as a substitute cereal for wheat. The percentages of wheat (X_W), rye (X_R), feed enzyme (X_E), and other ingredients (X_O) in a diet are the decision variables of the equation. In addition, the constraints of the equation are set as follows:

$$X_R + X_W = 60, \qquad (0 \le X_R \le 60); \qquad [5]$$

$$X_E + X_O = 40, \qquad (0 \le X_E \le 2); \qquad [6]$$

$$X_R + X_W + X_E + X_O = 100. \qquad [7]$$

When rewriting the Equations 5 and 6, the variables, X_W and X_O, can be represented by Equations 8 and 9,

$$X_W = 60 - X_R \qquad [8]$$

$$X_O = 40 - X_E \qquad [9]$$

Therefore, substituting Equations 8 and 9 into Equation 4, the optimum amounts of X_E and X_R for maximum profit can be calculated when the derivatives X_E ($\partial\Pi / \partial X_E$) and X_R ($\partial\Pi / \partial X_R$) of Equation 4 are equal to zero.

Analyses of Data

The parameters (A, B, C or a, b, c) of the log-linear model for weight gain or feed consumption for the data from the first and second study were calculated using a program developed by Zhang et al. (2000b). A multiple regression analysis was used for the data from the third study to establish the response of chick performances, such as weight gain (Y) and feed intake (F), to the amount of enzyme (X_E) and the proportion of rye (X_R) in the diet. The general regression equations are shown in the following equations (Equations 12 and 13).

$$Y = (A_0 + A_1X_R + A_2X_R^2 + A_3 X_R^3)$$
$$+ (B_0 + B_1X_R + B_2X_R^2 + B_3 X_R^3) \log (C X_E + 1) \qquad [12]$$

$$F = (a_0 + a_1X_R + a_2X_R^2 + a_3 X_R^3)$$
$$+ (b_0 + b_1X_R + b_2X_R^2 + b_3 X_R^3) \log (C X_E + 1) \qquad [13]$$

These models are an extension of the models used in our previous research (Zhang et al., 1996). The coefficients of the two regression equations, with the two variables such as the amounts of enzyme and the amount of rye relative to wheat in the diet as the inputs of the equation, were calculated by the stepwise regression method (SAS, 1994) where the C values of the log-linear equation were assumed to be 2,150. The data for the third study were also analyzed using Sigma Plot (Kuo and Norby, 1992) to determine the level of profit that was obtained with different amounts of an enzyme and different proportions of a substituted cereal, such as rye substituted for that of wheat, in a diet.

The standard error of means for all of the data are given in the original studies. The residual standard deviations of regression for the log-linear model equations are listed in Table 16.

RESULTS AND DISCUSSION

Evaluating the Effect of Different Feed Enzymes

One of the problems encountered by nutritionists in the feed industry is how to select a feed enzyme that would be most effective for a particular feed. The effect of different feed enzymes are generally evaluated by biological criteria such as their effect on chick performance, digestibility of feed nutrients, and degree of reduction of the viscosity of digesta or the diet (Bedford and Classen, 1992; Joroch et al, 1995; Zhang et al, 1996). In most studies, comparisons among different enzyme preparations have been often carried out using the same amount of different enzyme preparations in a cereal-based chick diet as determined by an enzyme activity assay or the levels of inclusion in the diet as recommended by the manufacturers (Rotter et al., 1989b; Guenter 1997b; Boros et al., 1998; Zhang et al., 2000b).

However, it is difficult to correctly evaluate different enzyme preparations based on their activities, since many enzyme preparations are from different sources. In addition, they often contain a different spectrum of enzymes with different catalytic properties. Therefore, the selection of the proper assay conditions such as pH, especially when comparing the activity of different feed enzymes, is essential because the selected pH will bias results in favour of an enzyme whose optimal pH is closest to the selected pH, which in turn may not be the optimal pH in vivo (Marquardt and Bedford, 1997; Ziggers, 1999, Zhang et al, 2000a, b). Recently, we have developed a new approach to accurately evaluate the effects of different enzyme preparations. The approach uses a new concept for estimating the efficacy of a feed enzyme, the slope of a log-linear model (Zhang et al., 1996). This evaluation, although very useful, is only based on the biological data. However, the goal of many studies is often to select an enzyme preparation that will yield the greatest profit.

The objective of the first study was to determine if the effects of different feed enzyme preparations on maximal profits could be evaluated using the MPEA. The profit functions (Table 16) were readily derived using Equation 4 together with the production (Equation 1) and feed consumption (Equation 2) functions. The maximal profit and the optimal amount of an enzyme that should be added to a diet were calculated using Equations 5 and 6. The results in Table 17 indicated that the maximum profits per 1000 birds when given the optimal amount of each of the five enzyme preparations, Cellulase Tv, Celluclast, Finizym, Cereflo, and SP249 were \$67.29, \$61.27, \$51.91, \$49.27, and \$46.79, respectively. In this analysis, the assumed price of the enzymes were the same. The sequence of these values also agrees with that of the B values for the feed to gain ratio as determined from the log-linear equation ($r^2 = 0.99$, P

TABLE 16. The production and feed consumption functions established from a log-linear model for the data from Zhang et al. (1996), Rotter et al. (1989), Marquard et al.(1994), and Bedford and Classen (1992)

Cereal[1] (Enzyme)	Y = Weight gain (g)[2]	r	SD[3]	F = Feed intake (g)[2]	r	SD[3]
Barley (CT)[4]	31.4 + 7.68 log (10^5 X +1)	.99	2.03	80.2 + 7.27 log (10^5 X +1)	.99	1.67
Barley (CC)	31.0 + 6.79 log (10^5 X +1)	.99	0.04	80.1 + 7.72 log (10^5 X +1)	.99	.66
Barley (FZ)	30.8 + 4.99 log (10^5 X +1)	.99	1.06	79.9 + 5.75 log (10^5 X +1)	.99	.28
Barley (CF)	30.3 + 4.49 log (10^5 X +1)	.92	3.21	79.3 + 4.61 log (10^5 X +1)	.93	3.28
Barley (SP)	30.6 + 4.00 log (10^5 X +1)	.96	1.91	79.5 + 4.63 log (10^5 X +1)	.94	2.94
Rye (RM1)[5]	536 + 45.6 log (10^3 X +1)	.99	7.3	1309 + 45.6 log (10^3 X +1)	.98	13.4
Rye (NQ)	549 + 52.2 log (10^3 X +1)	.98	13.1	1317 + 59.6 log (10^3 X +1)	.94	26.2
Corn (KC)[6]	135 - 1.67 log (10^4X +1)	.96	0.9	275 - 1.16 log (10^{10}X +1)	-.8	4.3
Wheat (KC)	125 + 0.81 log (10^{10} X +1)	.98	0.8	248 + 0.43 log (10^{10}X +1)	.90	1.1
Barley (KC)	113 + 2.26 log (10^{10} X +1)	.99	2.0	220 + 4.87 log (10^6 X +1)	.99	0.3
Rye (KC)	97 + 8.76 log (10^4 X +1)	.99	0.3	220 + 9.09 log (10^4 X +1)	.99	2.0
0% rye (PP)[7]	399 + 3.55 log (10^{10}X +1)	.81	11.4	664 + 13.1 log (10 X +1)	.52	10.0
20% rye (PP)	359 + 6.28 log (10^9 X +1)	.99	2.5	622 + 8.01 log (10^4 X +1)	.57	20.1
40% rye (PP)	306 + 21.6 log (10^5 X +1)	.98	9.2	561 + 30.8 log (10^4 X +1)	.93	21.0
60% rye (PP)	232 + 54.4 log (10^3 X +1)	.98	13.7	530 + 63.7 log (10^2 X +1)	.94	20.6

[1]Data from four studies were used to develop the prediction equation for weight gain, Y = A + B log (C X +1), and feed consumption, F = a + b log (c X + 1), where Y and F are weight gain (g) and feed consumption (g), X is the amount of an enzyme (%) added to a cereal-based diet, A, B, C and a, b, c are the coefficients of the log-linear equations for weight gain and feed consumption, respectively. The performances of chicks predicted by the equations were the values per bird when Leghorns were fed from 7 to 14 d (4: Rotter et al., 1989a) and from 7 to 21 d (6: Marquardt et al., 1994), and the values per 6 birds when Leghorns were fed from 7 to 21 d (5: Zhang et al., 1996), and when broilers were fed from 1 to 19 d (7: Bedford and Classen, 1992).

[2]The enzyme preparations used in the studies were RM1 and PP (a pentosanas preparation) from Finnfeed International Ltd; NQ and KC (Kyowa Cellulase) from Nutri-Quest; CT (Cellulase Tv concentrate) from Miles Laboratories Inc.; and CC (Celluclast), FZ (Finizym), CF (Cereflo), and SP (SP249) from Novo A/S Denmark.

[3]SD represents the residual standard deviation of regression for the log-linear equations.

TABLE 17. Effect of different enzyme preparations added to a barley- or rye-based diet on the efficacy of enzyme (B values), the optimal amounts of enzymes and the maximal profits obtained from Leghorn chicks in a one-week (Rotter et al., 1989a) or two-week (Zhang et al., 1996) feed study[1]

Source of Data	Enzyme Preparation[2]	B Value[3]	Optimal Enzyme[4] (%)	Maximal Profit ($/1000 birds)
Rotter et al., 1989	Cellulase Tv	-0.247	0.6557	67.29
	Celluclast	-0.2113	0.5603	61.27
	Finizym	-0.1716	0.4522	51.91
	Cereflo	-0.1386	0.4386	49.27
	SP249	0.1386	0.3849	46.79
Zhang et al., 1996	RM1	-0.0943	0.3152	101.19
	NQ	-0.0963	0.3481	104.38

[1] The assumed price for enzyme preparations used in the two studies was $5 per kg.

[2] The enzyme preparations used in the study of Rotter et al.(1989a) and Zhang et al. (1996) were Cellulase Tv concentrate (Trichoderma viride) from Miles Laboratories Inc. and Cellucast (*Trichoderma reesei*) Finizym (*Aspergillus niger*), Cereflo (*Bacillus subtilis*), and SP249 (*A. niger*) from Novo A/S Demark; and RM1 (*T. longibrachiatum*) from Finnfeeds International Ltd. and NQ (*T. reesei*) from Nutri-Quest.

[3] The B values were the slope of log-linear model equation calculated from the feed to gain ratio data. The values are the indexes of the efficacy of a feed enzyme added to a diet (Zhang et al., 1996; 2000a, b).

[4]Amounts of enzyme to yield a maximum profit.

< 0.0005). The same trend was also observed in this study using data from Zhang et al. (1996). These results, in contrast to subsequent results with different cereals or cereals plus enzymes, suggest that both the B values and the maximal profit provided similar indices for the evaluation of different enzyme preparations. This relationship, however, would not necessarily be the same if the price of the different enzyme preparations was different. In addition, the advantage of the two methods, especially for the method using maximal profit, is that they do not require a knowledge of enzyme activity, the combination of different enzymes used in a preparation, and the site of action of the enzymes in the gut. The information required for the latter method (the maximal profit) is that 1) the model equations be used to establish the influence of different concentrations of different enzyme preparations on chick performance and 2) the price of the major ingredients used in a diet. The method proposed in this study therefore provides a simple way to evaluate the collective economic effect of different enzyme preparations when incorporated into chick diets based on maximal economic returns.

Identifying the Most Profitable Cereal When Used With a Feed Enzyme

On the basis of the proposed method (Zhang et al., 1996), the most suitable cereal for a target enzyme preparation can be determined from the slope of a log-linear model. In this study (study 2), the B values for the feed to gain ratio were calculated from the data of Marquardt et al. (1994). The sequence of cereals producing the greatest response to an enzyme preparation in decreasing order were rye, barley, wheat, and corn (negative control) (Table 18). However, the sequence of the cereals that yielded the maximum profit following enzyme addition was different. The maximal profits obtained when the enzyme preparation

TABLE 18. Effect of cereal prices on optimal amounts of an enzyme added to different cereal-based diets and their maximal profits

Cereal	Value of B[1]	Same Price[2] ($/kg)	Optimal Enzyme[3] (%)	Maximal Profit ($/1000 birds)	Different Price[2] ($/kg)	Optimal Enzyme[3] (%)	Maximal Profit ($/1000 birds)
Corn	0.01	0.08	0	134.45	0.13	0	126
Wheat	-0.03	0.08	0.0339	133.55	0.12	0.0335	126.73
Barley	-0.04	0.08	0.0815	135	0.08	0.0815	135
Rye	-0.09	0.08	0.3419	123.96	0.08	0.3419	123.96

[1]The B values are the slope of log-linear model equation calculated from the feed to gain ratio data. The values are the indexes of the efficacy of a feed enzyme added to different diets. Therefore, the suitable cereal for a feed enzyme can be determined by this value (Zhang et al., 1996; 2000a, b).

[2]These values represent the price of the cereals. The assumption was that the enzyme (Kyowa Cellulase, Finnfeeds International Ltd.) Cost was $5 per kg.

[3]The optimal amount of enzyme calculated is that amount of enzyme that yield maximal profits.

was added to a barley-, corn-, wheat-, and rye-based diet were $135.00, $134.45, $133.55, and $123.96 per 1000 birds, respectively when the price of all cereals were the same (Table 18). Therefore, under these conditions, the relationship between the magnitude of the B values for feed to gain ratio and the maximal profit was low ($r^2 = 0.61$, $P = 0.2171$). This disagreement was also observed in the third study ($r^2 = 0.59$, $P = 0.2340$). The reason for this discrepancy is attributed to the fact that the B values reflect the overall response of chicks to different amounts of an enzyme added to different cereal-based diets while the maximal profits are not only affected by the efficacy of the enzyme (B value) but also by the response of chicks when fed different cereal-based diets without enzyme addition (A),

and by the cost of the feed and the enzyme. The results therefore demonstrate that the latter method (maximal profit) is more useful than the former procedure (B value) for the feed or enzyme industry in determinating which cereal should be used with a given feed enzyme to obtain the maximum profit. In addition, if the prices of wheat and corn were assumed to be $0.12 and $0.13 per kg, wheat yielded a greater profit than corn and the rye grain at $0.08 per kg becomes a competitive cereal with wheat or corn ($123.96 per 1000 birds for rye vs. $126.00 or $126.73 per 1000 birds for corn or wheat). These results demonstrate that the price of a cereal also influences profitability when a special feed enzyme is added to the cereal-based diet. Therefore, an acceptable price for a substituted cereal such as rye, when used with an enzyme, could be determined by comparing its maximal profit with that obtained by the use of the standard cereal such as wheat.

Optimal Amounts of Enzyme and Cereal That Should be Used in a Diet

One of the important applications of MPEA is to determine the amounts of an enzyme and

a cereal that should be used in the diet to obtain a maximum profit. Generally, the performance response of chicks fed a diet with increasing amounts of an enzyme is a hyperbolic saturated response pattern. It is well known that equal incremental amounts of enzyme when added to a diet results in diminishing incremental changes in chick performance (Friesen et al., 1991; Bedford and Classen, 1992; Marquardt et al., 1994, Zhang et al., 1996). However, this study demonstrates that the dose response of profit obtained with the addition of a feed enzyme yields a quadratic rather than a hyperbolic pattern. The results, as shown in Figures 10 and 11, indicate that the profit obtained with increasing amounts of a feed enzyme was increased to a certain point. After that, the profit decreased with increasing amounts of the enzyme (Figures 10 and 11). This point can be readily calculated using the MPEA. The results demonstrated that the optimal amounts of different enzyme preparations that should be added to a diet (Table 17) were considerably different for a given feed enzyme when added to different cereal-based diets (Table 18), and for a feed enzyme when added to a diet with varying the proportions of two cereals (Table 15). These results, however, demonstrated that there was the high correlation between the values of B for feed to gain ratio and the optimal amount of an enzyme that should be used in a diet ($r^2 = 0.99$, $P < 0.0005$ for the first study, Table 17; $r^2 = 0.92$, $P < 0.05$ for the second study, Table 18; $r^2 = 0.89$, $P < 0.06$ for the third study, Table 19). Therefore, the amount of an enzyme that should be used in a diet to obtain maximal profit increases with an increasing B value for the feed to gain ratio. In addition, the optimal amounts of the two inputs to obtain maximum profit can also be determined by the MPEA. This is shown by the arrows indicated in Figure 12. The two variables used in this study were variable amounts of enzyme and variable proportions of rye and wheat.

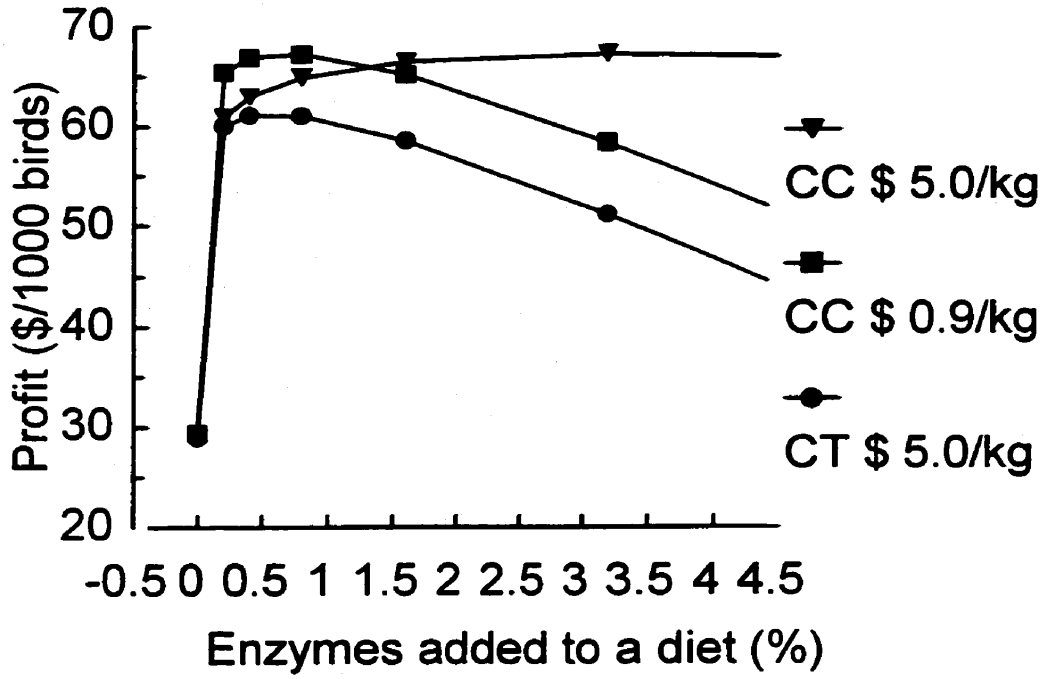

FIGURE 10. Estimated effect of the price of enzymes and the amount of enzymes added to a barley-based diet on the profit per 1000 birds in a one-week feeding study. The enzyme preparations used in this figure were CC (Celluclast) from Novo A/S Demark and CT (Cellulase Tv concentrate) from Miles Laboratories Inc. Data used for the calculations were from Rotter et al. (1989b).

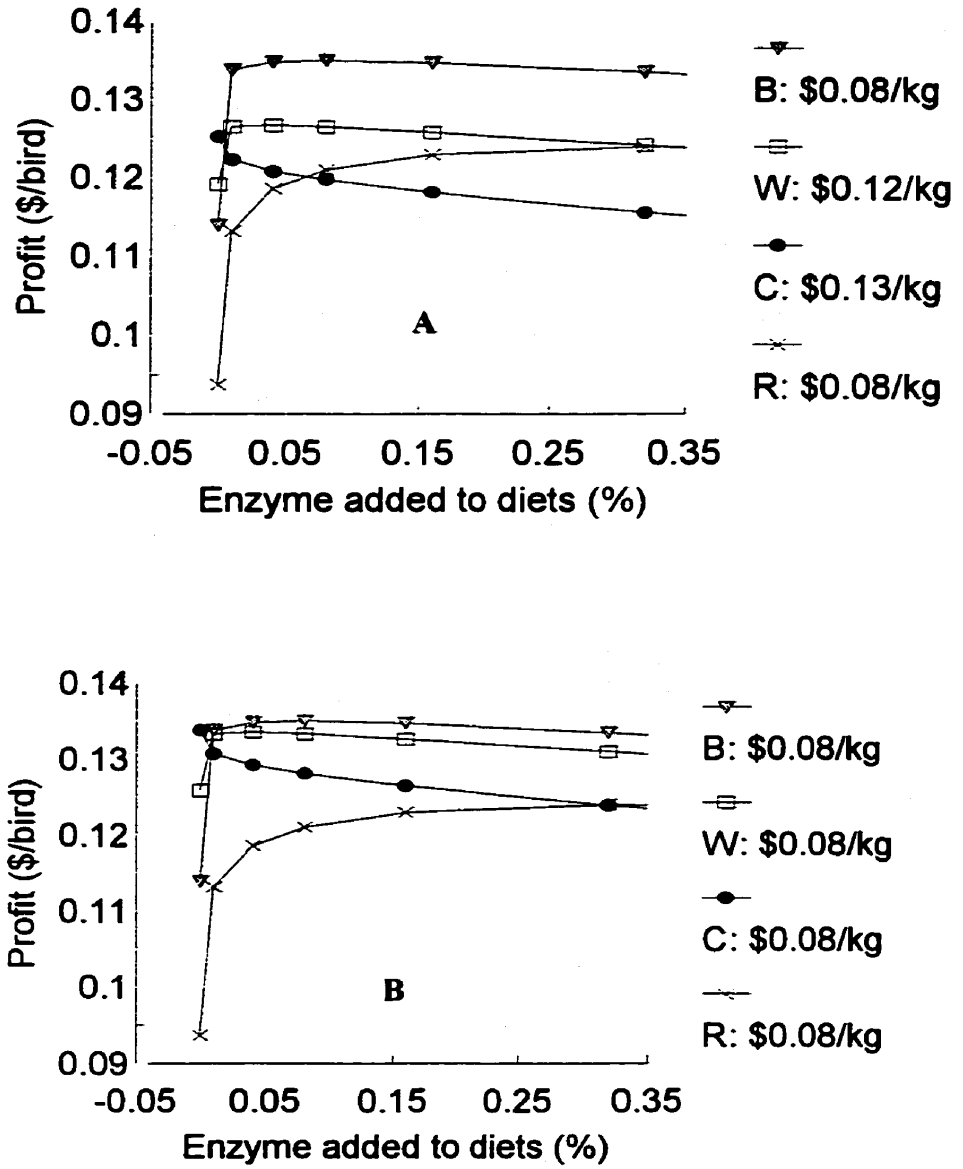

FIGURE 11. Profit as affected by cereal price and amounts of an enzyme added to the cereal-based diets. Different (A) and the same (B) price of cereals were used to calculate profit ($/bird). The assumed cost of the enzyme was $5/kg. Data used for the calculations were from Marquardt, 1994. C, corn; W, wheat; B, barley; and R, rye.

TABLE 19. Effect of price of an enzyme and cereals on the maximal economic returns and the optimal amounts of an enzyme to be added to diets with different proportion of rye

Price of Enzyme ($/kg)		$3		$5		$7	
Price Difference of Cereals[1] (Δ $ / kg)		$0.02	$0.04	$0.02	$0.04	$0.02	$0.04
Optimal enzyme		%					
	Rye 0 %[2]	0.273	0.273	0.159	0.159	0.113	0.113
	Rye 20 %	0.461	0.462	0.27	0.271	0.191	0.192
	Rye 40 %	0.738	0.742	0.435	0.438	0.309	0.311
	Rye 60 %	1.262	1.272	0.748	0.754	0.537	0.537
Maximal profit		$/1000 birds					
	Rye 0 %[2]	71.72	71.72	71.27	71.27	70.98	70.98
	Rye 20 %	68.15	68.59	67.4	67.83	66.91	67.34
	Rye 40 %	65.21	66.07	64.01	64.86	63.23	64.08
	Rye 60 %	63.27	64.58	61.19	62.48	59.85	61.13

[1]This price presents the net difference between the price of wheat and the price of rye that assumes a cheap cereal for substitution of wheat in diets.

[2]The corresponding amount of wheat in the four diets was 60, 40, 20, and 0%, respectively.

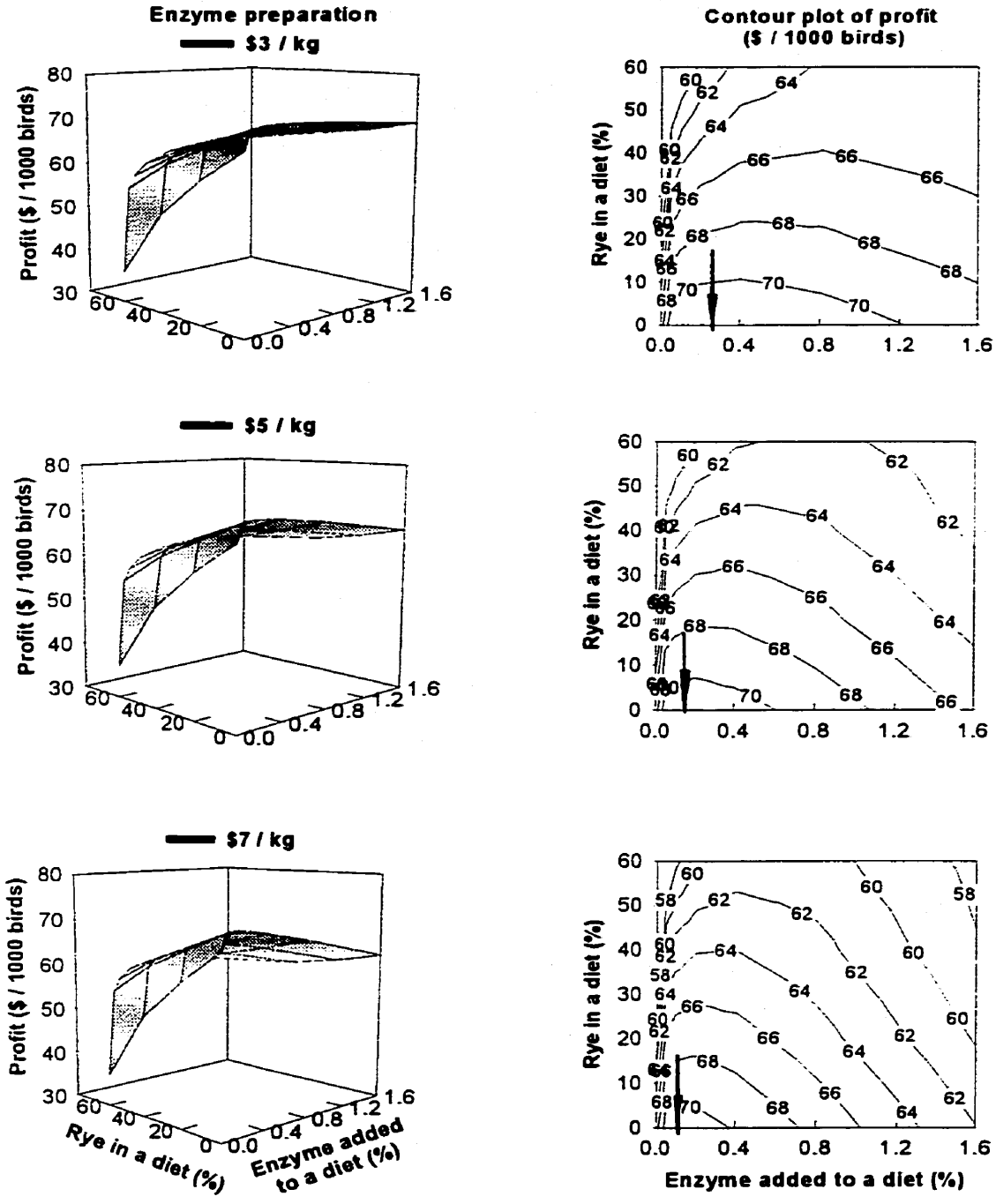

FIGURE 12. Effect of different combination of two variables, amounts of enzyme (X_E) and rye (X_R) added to diets on the profit of chickens fed diets from 1 to 19 d of age. Cereals in the diet were wheat plus rye (60 %). The profit function were: $\Pi = P_y\ Y - F \sum (Pi\ Xi)$,

where $Y = (404 - 2.04X_R + 5.25 \times 10^{-3} X_R^2 - 3.20 \times 10^{-4} X_R^3) + (9.78 + 3.49 \times 10X_R - 1.29 \times 10^{-3} X_R^2 + 1.06 \times 10^{-4} X_R^3) \log (2{,}150 X_E + 1)$, and $F = (648 - 8.52 \times 10^{-2} X_R^2 + 8.33 \times 10^{-4} X_R^3) + (2.64 + 2.27 \times 10^{-2} X_R^2 - 2.10 \times 10^{-4} X_R^3) \log (2{,}150 X_E + 1)$; Π = profit ($/1000 birds), Y = weight gain (g), F = feed consumption (g), Py and Pi represented the price of chickens and the price of the i-th ingredient (Xi) in a diet. The plots on the left gives the three-dimensional relationship for relative amount of rye in the diet (the balance is wheat), the amount of enzyme added to the diet, and the profits obtained assuming wheat and rye costs are $0.12 and 0.08 per kg, respectively, and that of enzyme is $3, 5, or 7 per kg. The figures on the right are the profit contours of two-dimensional slices of that on the right for diets containing different amounts of enzyme and different percentage of rye in the diet. The number in each line represents the fixed profit that can be obtained by feeding different amounts (%) of rye and enzyme. The arrow indicated the amount of enzyme that should be used to obtain maximal profits. Data used for the calculations were from Bedford and Classen, 1992.

Profit Contours or Isoquants

The objective of obtaining maximal profit by enzyme addition to a given diet may not be the only goal for a feed company or poultry farm. In some cases, the question that has to be asked is, does the enzyme and substituted cereal, used at various levels and in different combinations, give the expected profit? The relationships among amounts of enzyme added to the diet, the relative concentrations of two cereals (rye vs. wheat), the cost of the enzyme preparation, and the resulting profit are illustrated in Figure 12. The two-dimensional figures on the right side of Figure 12 represents the contour of response associated with horizontal slices of the figure on the left. These lines, called profit contours or isoquants, provide a useful tool to determine any combination of inputs such as amounts of an enzyme and rye used in a diet for any fixed level of profit. For example, if the price of enzyme was \$5 / kg (middle figure), it is possible to obtain \$65 (line labelled 65) of profit per 1000 birds with various combinations of rye (levels from 7 to 38%) and an enzyme (levels from 0 to 1.6 %). The arrows (↓) in the figures indicates the amount of enzyme that should be used to yield maximal profits. For example, maximum profits of \$71.72, \$71.27, or \$70.98 per 1000 birds was obtained when the enzyme content was 0.27, 0.16, or 0.11%, the rye content was 0%, and enzyme cost was \$3, \$5, or \$7 per kg, respectively. Different combinations of these inputs represent the optimal amounts of the enzyme and the rye that should be used in the diet to obtain maximum profits.

Decision Maker for Price of Enzyme and Cereal Used

There are many factors that influence the profit obtained when a feed enzyme is added to a diet. They included the amount of enzyme added, the type and amount of cereals, the

efficacy of the feed enzyme, and the price of enzyme and cereals used. Once the profit function is established, any variable in the equation can be calculated and analyzed when other variables are fixed. Therefore, the price that should be paid for an enzyme and a substituted cereal can be determined. This can be illustrated by the comparison of two enzyme preparations, Cellulase Tv concentrate (CT) and Celluclast (CC). The results in Table 17 indicated that the maximal profits per 1000 birds for CT and CC were $67.29 and $61.27 per 1000 birds when the price of both enzyme preparations was $5 per kg. If the maximal profit of $67.29 per 1000 birds was the target for both enzyme preparations, the price of CC should be reduced from $5 to $0.9 (Figure 10). The result suggest that the competitive price of CC can be determined by comparing its maximal profit with that of CT. Let us assume that CC was an enzyme preparation being developed to compete with CT for rye-based diets. Two possible methods could be used for CC in order to obtain the same maximal profit as that of CT. The first would be to reduce its price and the second would be to improve its efficacy (B value). The results indicated that an 82% decrease in the price of CC ($5 to $0.9) would be required to yield the same maximal profit ($67.29 /1000 birds) as obtained with CT at $5/kg. However, the same results could be obtained with a 17% improvement in the efficacy of CC, an increase in its B value for the feed to gain ratio from −0.211 to −0.247. This suggests that an improvement in the efficacy of an enzyme is a much more effective means of increasing profitability than that obtained by reducing its price. In some cases, it is not possible to obtain an equivalent maximal profit by changing the price of a feed enzyme. For example, the only way that Finizym could yield the same maximal profit as CT would be to improve its efficacy, i.e., improvement of its B value from −0.172 to −0.247 as shown in Table 17.

In addition, the maximal profit is also influenced by the price of the cereal used in a diet. As indicated in Table 18, rye grain cannot compete with wheat when the price of these cereal are the same. However, when a higher price of wheat was used, rye grain could yield a similar maximal profit to that obtained with wheat. This strategy could also be used by a feed industry to determine the expected price of a target cereal in order to obtain a certain level of maximal profit.

In conclusion, the MPEA that was developed in this study can be used by the enzyme and feed industries to evaluate different enzyme preparations based on their profitability, to determine the maximal economic return that can be obtained with the optimal inputs of feed ingredients such as type and amounts of cereals and enzymes, and to analyze the relationship between the price of a feed enzyme or a cereal and the economic return. This study demonstrates that a knowledge of nutrition in combination with computer technology and the modeling method can provide nutritionists and managers in the enzyme and feed industry with useful information for their research activities and business decisions.

MANUSCRIPT 4

EVALUATING THE EFFICACY OF ENZYME PREPARATIONS AND PREDICTING THE PERFORMANCE OF LEGHORN CHICKS FED RYE-BASED DIETS WITH A DIETARY VISCOSITY ASSAY

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ABSTRACT We studied whether a single-step dietary viscosity assay could be used to evaluate the efficacy of an enzyme when added to a poultry diet. The results demonstrated that there was a linear relationship between the log of the dietary viscosity change in vitro as determined using the new assay and the log of the enzyme activity (xylanase) added to a rye-based diet. The sensitivity of the dietary viscosity assay was high, as little as 0.19 U of xylanase per gram of diet could be detected. In addition, there was a high correlation ($r \geq 0.97$; $P < 0.005$) between chick performance and the log of the amount of enzyme added to the diet or the log of its viscosity change in rye-based diets that contained different amounts of xylanase as determined by the in vitro dietary viscosity assay. Further, the dose response data from the dietary viscosity assay, when incorporated into a log-linear model that we developed, was able to distinguish between the efficacy of two enzymes with regards to their ability to hydrolyze the viscosity factor in rye grain. Therefore, it was possible to accurately evaluate the efficacy of enzyme preparations in a rye diet and predict chick performance using the new assay in conjunction with a model equation.

(*Key words*: viscosity assay, enzymes, efficacy, chicks, rye-based diet)

INTRODUCTION

Exogenous enzymes have been shown to dramatically improve the utilization of certain feedstuffs, such as barley, wheat, rye and oats, especially in poultry (Fengler and Marquardt, 1988; Friesen et al., 1991; 1992; Bedford and Classen, 1992; Marquardt et al., 1994; Zhang et al., 1996; 1997). This effect is associated with reductions in intestinal viscosity of digesta (Antoniou et al., 1981; Hesselman and Aman, 1986; Fengler et al., 1988; Bedford and Classen, 1992; Graham et al., 1993; Marquardt et al., 1994; Zhang et al., 1996, 1997). Therefore, it should be possible to evaluate the efficacy of an enzyme preparation by its ability to degrade viscous compounds, such as arabinoxylan in rye and wheat and β-glucan in barley and oats, in pure target substrates, feedstuffs, diets and intestinal digesta.

Many problems in the use of enzymes need to be solved before the full potential of enzymes is reached. As far as we are aware, no satisfactory assay has been developed for monitoring the amounts of enzyme in diets and for evaluating the efficacy of an enzyme preparation. Part of the reason for this is that many different enzyme assays are used to monitor enzyme activity under different assay conditions (Marquardt and Bedford, 1997). Also, it is difficult to carry out enzyme assays in dietary ingredients as they not only are present at low concentrations, but are often bound tightly to feedstuffs (Bedford, 1993; Bernier et al., 1994; Tenkanen et al., 1995). As a result, activities are low and the enzyme is not quantitatively extracted from the diet. In addition, it is difficult to evaluate the efficacy of an enzyme added to a diet based on its activity. The efficacy of an enzyme in this study is defined as the ability of an enzyme, when added to a diet, to improve chick performance or reduce dietary viscosity within a certain period. [i.e., Δ chick performance or Δ dietary viscosity /Δ amount of enzyme /

testing time, where Δ is the mathematical term delta (change)]. Part of the difficulty is that the response of an exogenous enzyme is not usually linearly related with the amount of enzyme added to the diet, because the ability of an enzyme to improve chick performance will decrease with increasing amounts of enzyme added to a diet (Zhang et al., 1996).

Currently the procedures for the assay of most enzymes in diets involve extraction of enzyme from the diet prior to mixture with substrates, resulting, as discussed above, in low and variable yields of enzyme in the extract. A different approach would be to incubate an enzyme with its substrate in the presence of the dietary matrix, thereby exposing the substrate to both the free and bound forms of the enzyme. The hydrolyzed product can then be separated from the particulate matter and analyzed. This type of assay should provide a basis for the development of a more accurate method to assess the activity of an enzyme when present in the diet. A viscosity assay was developed by Bedford and Classen (1993). The assay, however, involved a two-step digestion procedure that was time consuming. They reported the relationship between viscosity change and animal performance but did not report the relationship between the amount of enzyme added to diet and the viscosity change. The correlation (r^2) that was obtained between the in vitro assay and improvement in animal performance indicated that the in vitro assay only had moderate accuracy ($r^2 = 0.67$). Therefore, there is a need to develop a simple one-step in vitro assay that not only predicts amount of enzyme in a diet but also can more accurately predict the relationship between activity of an enzyme preparation, as estimated from the in vitro assay, and animal performance.

There has been increasing interest in developing methods to accurately assess the efficacy

of an enzyme preparation in poultry diets (Zhang et al., 1996; Marquardt and Bedford, 1997). Recently, we developed a simple log-linear model equation [$Y = A + B \log (C X + 1)$] to accurately predict the response of poultry to a particular feed enzyme with the correlations (r values) between log amounts of enzyme added to the diet and performance values as high as 0.99 ($P < 0.001$). In this equation, Y is the estimated performance value [i.e., weight gain (g)]; X is the amount of enzyme in the diet (U/kg); A is the intercept, which represents the performance without enzyme supplementation; C is the adjusted factor; and B, the slope of the equation, is a measure of the efficacy of enzyme. Therefore, the value of B in the equation simply provides a new means to assess the efficacy or quality of an enzyme preparation when added to the diet. The value of B represents the increase of performance per log unit of an enzyme added to the diet of interest during a certain experimental period (Δ performance improvement /Δ log of enzyme activity / a certain experimental period) when fed to a certain class or type of poultry (Zhang et al., 1996; unpublished data). Furthermore, it is proposed that the log-linear model can be used, in combination with an in vitro dietary enzyme assay, to determine the amounts of an enzyme added to the diet and to assess accurately the efficacy of an enzyme.

The objectives of this paper were 1) to develop a simple in vitro dietary viscosity assay for estimating enzyme activities in the diet, 2) to determine if the activity obtained from the assay accurately predicts the actual amount of enzyme added to the diet, and 3) to demonstrate that the enzyme activity, when used in conjunction with the model equation (Zhang et al., 1996, unpublished data), can predict performance of chicks fed a highly viscosity diet. The diet would contain different amounts of enzyme to accurately assess the efficacy of an enzyme.

Therefore, the in vitro assay is designed to not only predict enzyme activity but also predict chick performance.

MATERIALS AND METHODS

Enzyme Sources and Diets

We used two enzyme preparations in this study, RM1 (a test sample[1]) and NQ.[2] The xylanase activity of the two enzyme preparations was 389 and 778 U/g of enzyme preparation, respectively, as determined by the azo-dye method of McCleary (1992) at pH 4.7. Rye grain was selected as one of the cereals because it contains high levels of viscous arabinoxylans (Antoniou et al., 1981; Fengler and Marquardt, 1988a; Marquardt et al., 1994; Zhang et al., 1996, 1997). The diet consisted of the following ingredients: 60% rye, 8.25% wheat, 24.5% soybean meal, 2.4% vegetable oil, and 4.35% other ingredients (Zhang et al., 1996). The final calculated protein concentration and MEn of the diets were 180 g/kg and 2,948 kcal / kg diet, respectively, which met the requirements of National Research Council for Leghorn chicks (NRC, 1994).

The Feeding Study

A total of 432 1-d-old Single Comb White Leghorn cockerels were fed a commercial starter diet for a 7-d pre-experimental period and after 4 h of food deprivation were randomly distributed in experimental units in such a way that all units had the same average initial

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[1]Finnfeeds International Ltd. Wiltshire, UK SN8 1AA.

[2]Nutri-Quest, Chesterfield, MO 63017.

weight. Six different amounts of two enzymes, RM1 (0, 0.25, 0.75, 2.25, 6.75, and 20.25 g/kg) and NQ (0, 0.1, 0.3, 0.9, 2.7, and 8.1 g/kg), were added to the diet for a total of 12 treatments. The corresponding activity values of each enzyme were similar (Table 20). Each treatment consisted of six replicates per treatment with six birds per replicate (an experimental unit). The experimental diets were fed to birds from 7 to 21 d of age. Weight gain and feed consumption were recorded 4 h after removal of feed at 14 and 21 d of age. In the course of the experiments, chicks were kept in the same Petersime batteries with constant light and heat and had free access to water and feed. The research protocol was approved by the local animal care committee and followed the guidelines of the Canadian Council of Animal Care.

In Vitro Dietary Viscosity Assay

Enzyme preparations (NQ or RM1) were dissolved in 0.2 *M* phosphate buffer, pH 4.8, for 1 h at room temperature and centrifuged at 3000 X *g* for 15 min. Different concentrations (0, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6%) of the two enzymes were added as extracts to the rye diet without enzyme addition, and were used for the development of the in vitro dietary viscosity assay. However, the diets that had been supplemented with the powdered enzymes for the feeding studies were used for subsequent studies to establish the relationships among performance of chickens, enzyme activity added to the diet, and dietary viscosity change measured by the in vitro viscosity assay.

Preliminary Studies. The first study was conducted to establish the influence of the concentration of the buffer and its pH on the pH and viscosity values of the dietary extract (4 mL / g diet). Solutions that contained 0, 0.2, 0.4, 0.6, 0.8, and 1.0 *M* phosphate buffer at pH 5.0, 6.0, and 7.0 were compared. In a second study, the pH of the extracting buffer

TABLE 20. Effect of two enzyme preparations (RM1 and NQ), when added to the rye-based diet, on the performance of Leghorn chickens and the log of dietary viscosity as determined by the in vitro viscosity assay[1]

Enzyme preparations		Weight gain (g per 6 birds)		Feed to gain ratio (g / g)		Log dietary viscosity (log centipoise)	
Sources[2]	U/kg	Wk 1	Wk 1+2	Wk 1	Wk 1+2	4 h	8 h
RM1	0	193^{f}	543^{e}	2.65^{a}	2.43^{a}	0.591^{a}	0.527^{ab}
	97	248^{de}	601^{d}	2.28^{bc}	2.29^{b}	0.548^{c}	0.511^{ab}
	292	256^{cde}	611^{dc}	2.25^{bcd}	2.27^{bc}	0.531^{c}	0.489^{bc}
	875	268^{bcd}	640^{bcd}	2.18^{cde}	2.19^{de}	0.469^{d}	0.440^{d}
	2626	275^{bc}	672^{ab}	2.17^{cde}	2.17^{e}	0.422^{e}	0.369^{e}
	7877	284^{b}	690^{ab}	2.09^{de}	2.11^{f}	0.349^{g}	0.278^{f}
NQ	0	199^{f}	539^{e}	2.62^{a}	2.42^{a}	0.560^{ab}	0.538^{a}
	79	236^{e}	604^{d}	2.37^{b}	2.29^{b}	0.551^{bc}	0.482^{bcd}
	233	275^{bc}	646^{bcd}	2.16^{cde}	2.21^{cd}	0.485^{d}	0.461^{cd}
	700	284^{b}	657^{abc}	2.07^{e}	2.18^{de}	0.391^{f}	0.344^{e}
	2100	277^{bc}	661^{abc}	2.13^{cde}	2.15^{de}	0.257^{h}	0.182^{g}
	6302	308^{a}	701^{a}	2.06^{e}	2.16^{de}	0.111^{i}	0.081^{h}
Pooled SEM		7	16	0.05	0.03	0.0002	0.0004
Overall CV		2.7	2.5	2.2	1.3	0.05	0.1

[a-c]Means in each columns not having the same superscript are significantly different ($P < 0.05$).

[1]The data for chick performance are from the original data of Zhang et al. (1996). Copyright permission was obtained from Journal of Animal Science. The values are means; n = 6 for performance of chickens, and n = 2 for dietary viscosity assay.

[2]RM1 (Finnfeed International Ltd., Witshire, UK) and NQ (Nutri-Quest, Chesterfield, MO) contained 389 and 778 U of xylanase activity / *g* of original enzyme preparation (stock enzyme), respectively, as determined by the colorimetric method of McCleary (1992) at pH 4.7. The stock preparations were added to the diets to give the indicated activity values.

(0.2 *M* phosphate) that was required to give final extract pH of 5.0, 6.0, and 7.0 was determined. A third study established the effect of incubation time (Figure 13) and pH (Figure 14) of buffered suspensions of the rye diet, with and without the enzyme, on the final viscosity of the extract. In these studies, the rye diet (1 g), without and with 0.1% NQ, was extracted for 0, 1, 2, 4, 8, 16, and 32 h with 4 mL of 0.2 *M* phosphate buffer having pH values of 4.8, 6.4, and 7.5. The extracts were assayed for viscosity using the procedures described in the following section. The net effect of the enzyme was determined by subtracting the logarithmic viscosity values obtained in the presence of an enzyme from those obtained in the absence of an added enzyme. The logarithmic transformation was used because the amount of viscous arabinoxylans that are extracted from rye are directly proportional to the log of their viscosity values (Boros et al., 1993). The final pH of the three extracts was 5, 6, and 7. All extracts were prepared in duplicate.

Assay Procedure. The extraction procedures were developed on the basis of results obtained in this paper. The method for viscosity measurement was a modification of the procedures of Boros et al. (1993). One gram of a diet for the viscosity assay was finely ground (passed through a 1 mm mesh) and mixed with 4 mL of 0.2 *M*, pH 4.8, phosphate buffer in duplicate; the suspension was vigorously shaken at 250 rpm at 40 C in a Controlled Environment Incubator Shaker[3] for a fixed incubation time, i.e., 1, 2, or 8 h. It was then centrifuged for 15 min at 13,000 X *g* at 4 C, and the supernatant was stored until assayed in an ice bath to minimize further enzymatic activity. The final pH of the extracts was 5.0 ± 0.1.

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[3]New Brunswick Scientific Co., Inc., Edison, NJ 08818-4005.

[4]Brookfield Engineering Laboratories, Inc., Stoughton, MA 02072.

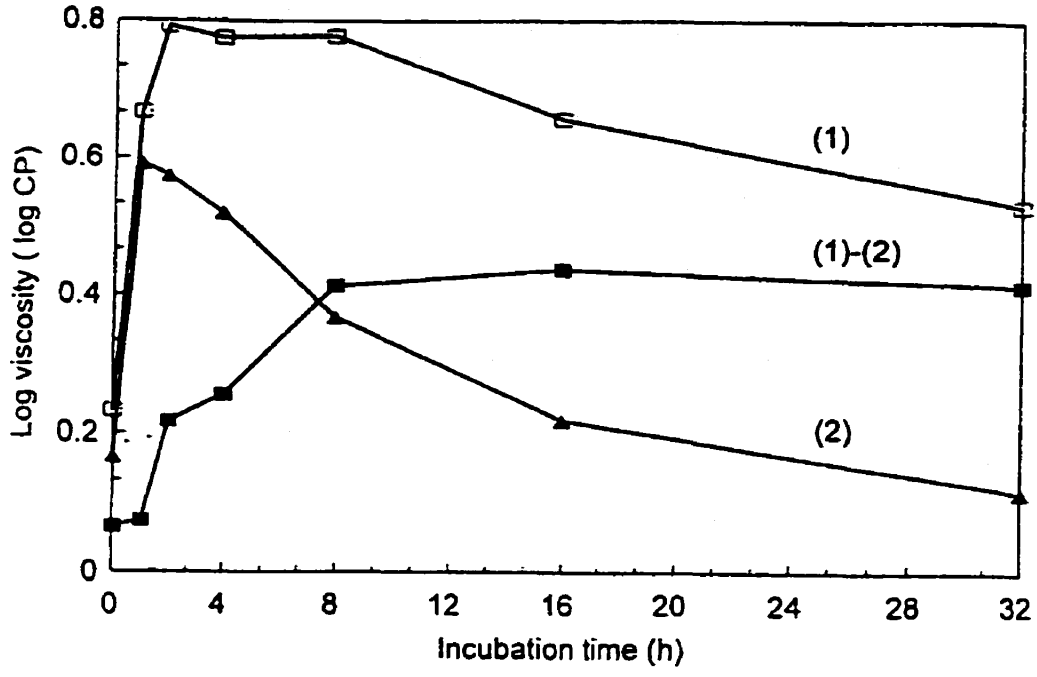

FIGURE 13. Kinetics of in vitro dietary viscosity change of a rye-based diet without [(1), -□-] and with [(2), -▲-] enzyme supplementation (0.1% NQ, Nutri-Quest, Chesterfield, MO). The differences [(1)-(2), -■-] between the two values represents the net effect of the enzyme added to the diet on dietary viscosity change. Viscosity changes were assayed at 40 C over 32 h and expressed as log centipoises (log CP). Overall standard errors for the data without and with enzyme were 0.011 and 0.017 log CP, respectively (n = 2). Extracts of the diet were prepared in 0.2 *M* phosphate buffers pH 4.8 (final extract pH = 5.0).

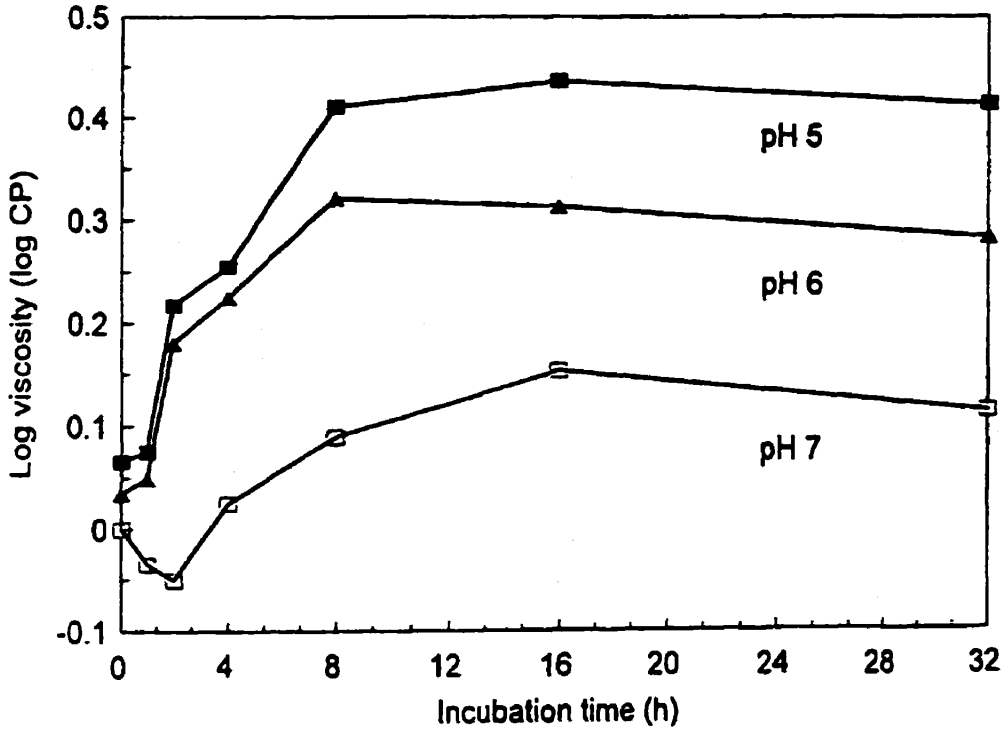

FIGURE 14. Kinetics of net log of dietary viscosity change [log centipoises (log CP)] of a rye-based diet supplemented with a crude fungal enzyme preparation (0.1% NQ, Nutri-Quest, Chesterfield, MO). The values represent the differences in log of viscosity change (log CP) between the unsupplemented and supplemented diets with enzyme following incubation at pH 5(-■-), 6(-▲-) and 7(-□-) for different periods of time at 40 C. Overall standard errors for the pH 5, 6, and 7 data were 0.017, 0.014, and 0.028 log CP, respectively.

A 0.5-mL aliquot of the extract was transferred into the viscometer chamber. The results were recorded at 1 min of operation. Viscosity measurements were carried out at 25 C using a Wells-Brookfield Cone/Plate Digital Viscometer, model LVT DV-1CP[4], with a 0.8 inch cone spindle at a shear rate of 4.5 to 450 per second. All results are reported in centipoises (CP = dyne s / cm^2). The extracts were assayed for viscosity within 1 h after centrifugation. Preliminary studies demonstrated that under the conditions of the assay no change in viscosity occurred over this time period.

Analyses of Data

Data from the feeding trial and the in vitro dietary viscosity assay were analysed by ANOVA to determine significance of main effects. Duncan's multiple range test was used for multiple comparisons of means ($\alpha = 0.05$; SAS, 1988). The means of the data were also subjected to regression analysis. A completely randomized design was used for the feeding trial with each cage being experimental unit.

The parameters for the log-linear model were calculated using a specific computer program (Zhang et al., 2000b). Comparisons with the values of B (NQ vs. RM1) as calculated from the output values, such as log viscosity change (measure enzyme activity) as determined by the in vitro dietary viscosity assay developed in this study or the chick performance, and the input values, such as enzyme activities expressed as the amounts added to the diet, were made using a *t*-test with $\alpha = 0.05$.

RESULTS AND DISCUSSION

Development of an In Vitro Dietary Enzyme Assay

Previous studies have demonstrated that hydrolysis of the arabinoxylans in rye and wheat and β-glucans in oats and barley can result in a dramatic reduction in viscosity of digesta that is related to the activities of the added enzyme(s) and the corresponding improvements of the nutritional value of the diet (Hesselman et al., 1982; Annison and Choct, 1991; Campbell and Bedford, 1992; Chesson, 1993; Bedford, 1995). One of the objectives of this study was to develop and optimize conditions for the detection of enzymes when added to rye-based diets using a simple in vitro viscosity assay. This assay should be a useful approach because, as indicated above, one of the main functions of exogenous enzymes is to reduce digesta viscosity. The principle of the assay was to allow the enzyme in the diet to interact with the substrates in a buffered suspension of the diet for an appropriate time period followed by centrifugation of the suspension and the measurement of the viscosity of the solution. The logarithmic value of the control diet without enzyme supplementation less the logarithmic value of the diet with enzyme supplementation is the net viscosity change due to the presence of the enzyme. This difference provides an indication of the net relative amount of the substrates, such as viscous arabinoxylans, that are hydrolysed (Boros et al., 1993).

Optimization of pH and Concentration of Extracting Buffer. A preliminary study suggested that lower concentrations of the pH 5.0 or 6.0 phosphate buffers (0.2 or 0.4 *M*) were sufficiently high in buffering capacity to control the pH of an extract of the diet and yield maximal viscosity reductions when the diet was incubated with enzyme. Higher ionic concentrations of buffer should not be used as they have an inhibitory effect on enzyme activity (Tenkanen et al., 1995; Zhang, unpublished data). Phosphate buffer at a concentration of 0.2 *M* was selected as the extracting buffer because it best met both criteria. In a second

study it was shown that the pH of the 0.2 *M* phosphate extracting buffer (4 mL/g of diet) should be 4.8, 6.4, or 7.5 to yield an extract pH of the diet of 5, 6, and 7, respectively.

Incubation Time. The time course changes in the log of viscosity of a buffered suspension (final pH = 5) of a rye diet without and with 0.1% NQ are illustrated in Figure 13. Approximately 1 and 2 h were required to reach maximal viscosity values with and without the enzyme after which there was a time dependent decline in viscosity of both extracts, with the rate of reduction being more rapid for the sample containing added enzyme. The initial increase in viscosity over a 2-h period is probably a reflection of the amount of the soluble and highly viscous arabinoxylan that is being extracted from the rye (Boros et al., 1993). The decline in viscosity over time for both diets can be attributed to the combined effects of endogenous plus added exogenous enzymes in the rye-based diet and the effects of the endogenous enzymes alone in the rye diet. The difference between the two values therefore represents the net change due to the presence of the added enzyme. In addition, the kinetics of the viscosity change for extracts prepared from the diet without enzyme supplementation clearly demonstrated that there was a period of 2 to 8 h during which the viscosity values remained relatively constant. The endogenous viscosity-reducing enzymes, however, were also able to significantly reduce the viscosity values of the extract over longer periods of time (8 to 32 h). In contrast, the exogenous enzymes dramatically reduced the viscosity of the extracts after 1 h. The results indicated that near-linear net logs of viscosity changes were obtained within 8 h indicating that a time period between 1 to 8 h would be most suitable for monitoring enzyme activity. The time period of 4 h was selected so as to maximize viscosity changes and therefore the precision of the assay.

Figure 14 depicts the net change in log of viscosity when the assays were carried out at three different pH values. The greatest absolute change in the log of viscosity occurred at pH 5, followed by pH 6 and 7. The rate of viscosity change also was the greatest at pH 5, intermediate at pH 6 and much slower at pH 7. The selection of the appropriate assay pH, especially when comparing the efficacy of different enzyme preparations, is essential because the selected pH will bias results in favor of the enzyme whose optimum pH is closest to the selected pH. The strategy of selecting the assay pH should consider two points. First, the assay pH should reflect the environment where the enzyme acts in the gut. However, it is not known whether the main site of action of exogenous enzymes in chickens is in the crop, proventriculus, gizzard, duodenum, ileum, rectum, or all or part of the gastrointestinal tract (Marquardt and Bedford, 1997). Second, the sensitivity and accuracy of the assay should also be considered. In this regard, most enzymes of fungal origin show a pH-optimum in a range of 2.5 to 5.0 (van de Mierop and Ghesquiere, 1998). The two enzyme preparations in this study, RM1 and NQ, were from the same species of fungus, *Trichoderma longibrachiatum* or *T. reesei*. The xylanase activities of the two enzymes at pH 5.3 and 6.0 were 3,450 and 7,700 U/g for RM1 , and 2,250 and 6,000 U/g for NQ, respectively, as assayed by Finnfeed International using the 3,5-dinitrosalicylic acid reducing sugar method. These data plus our preliminary data (not shown) indicate that the optimal pH for xylanases was approximately 5.0. Considering these two points, a pH of 5.0 was selected as the assay pH for routine analysis because it yielded the maximum rate of viscosity change, whereas the pH at which the enzyme is effective in the intestine was not considered as it is unknown. This pH, however, is not greatly different than the pH of digesta in the crop (4.5 to 6.5), but is lower

than that of the duodenum (5.8 to 6.5) (Duke, 1986; van de Klis et al., 1993b; Danicke et al., 1997; Boros et al., 1998). However, further studies need to be carried out to identify the pH that reflects the in vivo pH at which the enzyme is most effective.

Standard Curves. The objective of the final study was to establish the relationship between dietary viscosity change and the amount of enzyme added to the diet and to determine the incubation time that most accurately predicts the amount of enzyme in the diet (Figure 15). Viscosity was expressed as its log value because this yields values that are equivalent to the amount of substrate hydrolyzed (Boros et al., 1993). The results demonstrate that there was a linear relationship ($r > 0.99$, $P < 0.005$) between log of the net viscosity change from the dietary extracts and log of the concentration of enzyme added to the diet for all three incubation times (1, 2, and 8 h, respectively). Using this relationship with the appropriate references (enzyme and diet) as standards, it should be possible to determine the amount of an enzyme in a diet using the in vitro viscosity assay. In the current assay, the smallest amount of the enzyme that could be detected was 0.025% NQ (0.19 U of xylanase activity /g of diet) after 2 h of incubation, whereas the lowest amount of xylanase that has been reported to be detectable with the radial diffusion assay appears to be much higher and requires a much longer assay time (Walsh et al., 1995). Therefore, this assay appears to be superior to the radial diffusion assay, because much lower activity values can be assayed, and the assay time is much less.

Relationships Between Determined and Actual Enzyme Activities in a Diet

Zhang et al. (1996) demonstrated that there was a linear relationship between chick performance and the log amounts of enzyme added to a diet. The results of Bedford and

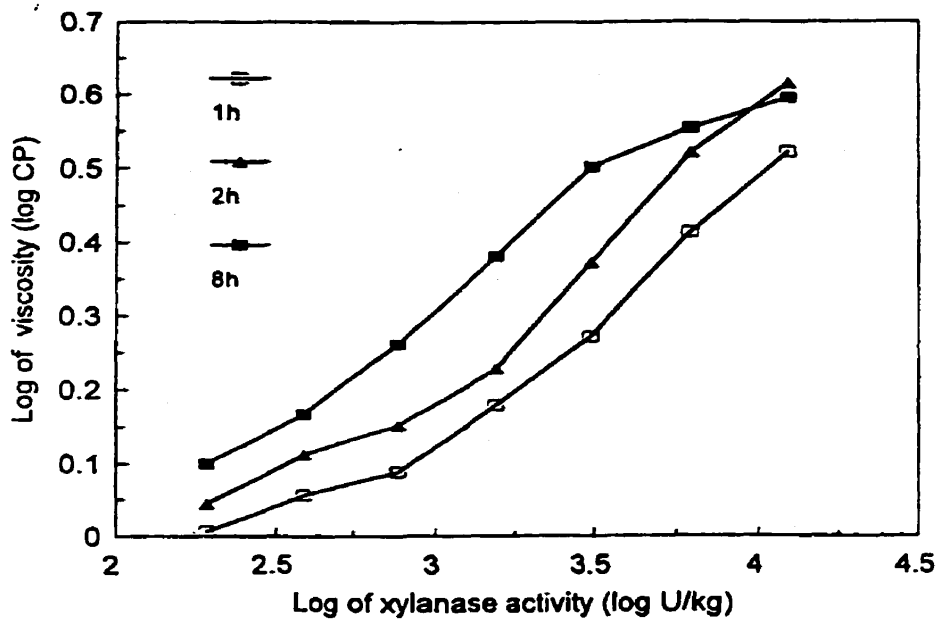

FIGURE 15. Log of viscosity changes [net log centipoises (log CP)] as affected by the concentration of the enzyme (% NQ, Nutri-Quest, Chesterfield, MO) in extracts prepared from a rye diet containing different concentrations of the enzyme. The extracts were incubated using the standard assay for different time intervals (-□-, 1 h, -▲-, 2 h and -■-, 8 h) at 40 C, and viscosity values were converted to their logarithmic values and subtracted from the control values (diets with no enzyme supplementation) to yield net viscosity values. Overall average standard errors for the1, 2 and 8 h data were 0.009, 0.022, and 0.007 (log CP), respectively.

Classen (1992) indicated that there was a linear relationship between chick performance and the log of intestinal viscosity. Therefore, we hypothesized that a linear relationship exists between the log of viscosity change, as determined by the in vitro assay and the log amounts of enzyme added to a diet. Such a relationship was established in this study (Table 21, log CP vs. log U/kg, $r > 0.99$, Figure 15). The same trend was obtained for all of the different assay times (1 to 8 h, data not shown). The use of a model equation (Zhang et al., 1996) to establish the nature of this relationship is discussed subsequently. The results in Table 22 further demonstrate that the in vitro assay accurately estimated the amount of enzyme in the diet when viscosity was converted into its logarithmic value but not when its arithmetic value was used ($r < 0.82$, $P > 0.05$). The data in Table 22 that were used to calculate the coefficients of correlation (r) between chick performance and the dietary viscosity change were from Table 20. Likewise, the amount of the enzyme in the diet, when expressed as log of its activity value (U/kg diet), was more closely related to viscosity changes in diet extracts when converted to their log values as compared with their arithmetic values (Zhang et al., unpublished data). Collectively these results demonstrate that when the viscosity values from the in vitro assay are converted into their log values, they can accurately predict amount of enzyme in a diet with highly viscous compounds. The limitations of this assay and methods of improving it are discussed subsequently. Bedford and Classen (1993) also developed an in vitro assay but it has the limitations that were discussed in the Introduction.

Prediction of Chick Performance by the In Vitro Dietary Viscosity Assay

The objective of this comparison was to determine if data from the in vitro viscosity assay could be used to accurately predict chick performance and to determine if its predicted value

TABLE 21. Use of a log-linear model equation to evaluate the efficacy of enzymes added to the rye based diet by in vitro dietary viscosity assay and predict chick performance from the enzyme activity expressed as the amounts added to the diet (AEA) or the viscosity change determined by an in vitro dietary viscosity assay (MEA)[1]

Output (Y)	Input (X)	Parameters obtained from prediction equations				
		A	B[4]	C	r	$P <$
Viscosity[2] vs. AEA[3]						
MEA (log CP)	RM1[5](log U/kg)	−0.02	0.124[a]	10^{-2}	0.99	0.005
	NQ[5] (log U/kg)	−0.03	0.265[b]	10^{-2}	0.99	0.005
Performance vs. AEA						
WG1 (g/6 birds)	RM1 (log U/kg)	195	18.0[a]	10	0.99	0.005
	NQ (log U/kg)	191	22.3[a]	10	0.95	0.005
FG1 (g/g)	RM1 (log U/kg)	2.64	−0.091[a]	10^{2}	−0.99	0.005
	NQ (log U/kg)	2.65	−0.127[a]	10	−0.95	0.005
Performance vs. MEA[3]						
WG1 (g/6 birds)	RM1 (log CP)	198	69.5[a]	10	0.99	0.005
	NQ (log CP)	198	72.2[a]	10	0.97	0.005
FG1 (g/g)	RM1 (log CP)	2.65	−0.24[a]	10^{2}	−0.99	0.005
	NQ (log CP)	2.65	−0.25[a]	10^{2}	−0.98	0.005

[1]WG1 and FG1 were actual weight gain and feed-to gain ratio over a 7-d period of a feeding trial for chicks fed a rye-based diet containing different amount of added enzyme (RM1 or NQ). The relationship between the outputs, such as chick performances (WG1 and FG1) or log of dietary viscosity change [log centipose (CP)], and the inputs such as the amount of enzyme in the diet (AEA or MEA), was estimated from a prediction equation (Zhang et al., 1996, unpublished data). The equation is $Y = A + B \log (C X + 1)$, where Y = outputs (WG1 and FG1 or MEA), and X = inputs, i.e., the amount of enzyme (xylanase) added to the diet (AEA) or its estimated activity value (MEA). The A is the intercept and represents performance without enzyme added to the diet. The B, the slope of the equation, represents the performance of chick per log unit of enzyme (weight gain or feed to gain ratio per log unit of enzyme for AEA, or weight gain or feed to gain ratio per log viscosity change for MEA, or log dietary viscosity change per log of enzyme activity added to the diet). The C is an adjusted factor that is required to calculate performance of chicks when fed diets

without added enzyme. The data used to calculate the parameters of the equations were from Table 20.

[2]The outputs are log of dietary viscosity changes (log CP, centipoise) obtained using the in vitro viscosity assay during a 4-h incubation period and the inputs are the log of the amount of enzyme added to the diet (log U/kg).

[3]The outputs (Y) are animal performances and the inputs (X) are units of enzyme activity added to the diet (AEA) or the enzyme activity as determined by in vitro dietary viscosity changes (MEA).

[4]B values for each comparison of the two enzymes not having the same superscript are significantly different ($P < 0.05$, t-tests).

[5]RM1 (Finnfeed International Ltd., Wiltshire, UK) and NQ (Nutri-Quest, Chesterfield, MO) contained 389 and 778 U of xylanase activity / g of the original enzyme preparation (stock enzyme), respectively, as determined at pH 4.7 by the colorimeteric method of McCleary (1992). The stock preparations were added to the diet to give the indicated activity values.

TABLE 22. Coefficients of correlation (r) between chick performance and the dietary viscosity change when the diet was incubated in vitro for 4 h

Chick performance[1]	Enzyme preparation[2]	Dietary viscosity change (centipoise)		Log of dietary viscosity change (log centipoise)	
		r	*P*	r	*P* <
WG1	RM1	0.88	0.021	0.97	0.005
	NQ	0.88	0.019	0.99	0.005
FG1	RM1	-0.82	0.045	-0.98	0.005
	NQ	-0.85	0.031	-0.99	0.005

[1]WG1 and FG1 represent weight gain and feed to gain ratio over a 1-wk period, respectively.

[2]RM1 (Finnfeeds International Ltd., Wiltshire, UK) and NQ (Nutri-Quest, Chesterfield, MO) contained 389 and 778 U of xylanase activity / g of the original enzyme preparation (stock enzyme), respectively, as determined at pH 4.7 by the colorimeteric method of McCleary (1992). The stock preparations were added to the diet to give the indicated activity values.

was as accurate as that obtained using known amounts of enzyme that were added to the diet. A model equation, developed by Zhang et al. (1996, unpublished data), was used to establish the nature of the relationship between amount of enzyme in the diet and chick performance. The previous study demonstrated that there is a linear relationship [$Y = A + B \log (C X + 1)$] between chick performance and the concentration of enzyme added to the diet when enzyme concentration is transformed into its log value. The equation predicts chick performance (Y) from the log of enzyme activity (X) when added to the diet. The data in Table 21 (chick performance vs. the enzyme activity added to the diet or measured enzyme activity by the in vitro assay) demonstrate that both the in vitro assay and the amount of enzyme added to the diet (the input, X) were highly correlated ($r > 0.95$) with animal performance (the output, Y). In addition, data in Figure 16 show that the amount of enzyme added to the diet (C and D) and the amount of enzyme as determined by the log of the in vitro viscosity assay (A and B) were linearly ($r > 0.99$, $P < 0.005$) related to weight gain (A and C) and the feed to gain ratio (B and D). These data demonstrate that the degree of improvement obtained when a given enzyme is added to the diet is a linear function of the log of enzyme activity which in turn is directly related to the log of the net dietary viscosity change as determined by the in vitro assay. Therefore, the log of dietary viscosity change not only was able to estimate the enzyme activity added to the diet, but also was able to predict the performance of chickens. Similar trends in r values were obtained when viscosity measurements were carried out at other time periods (8 h) and when chick performance was determined over a 2-wk period (Zhang et al., 2000b, c). These results further support the conclusion that the model equation as developed by Zhang et al. (1996, 2000b) can accurately

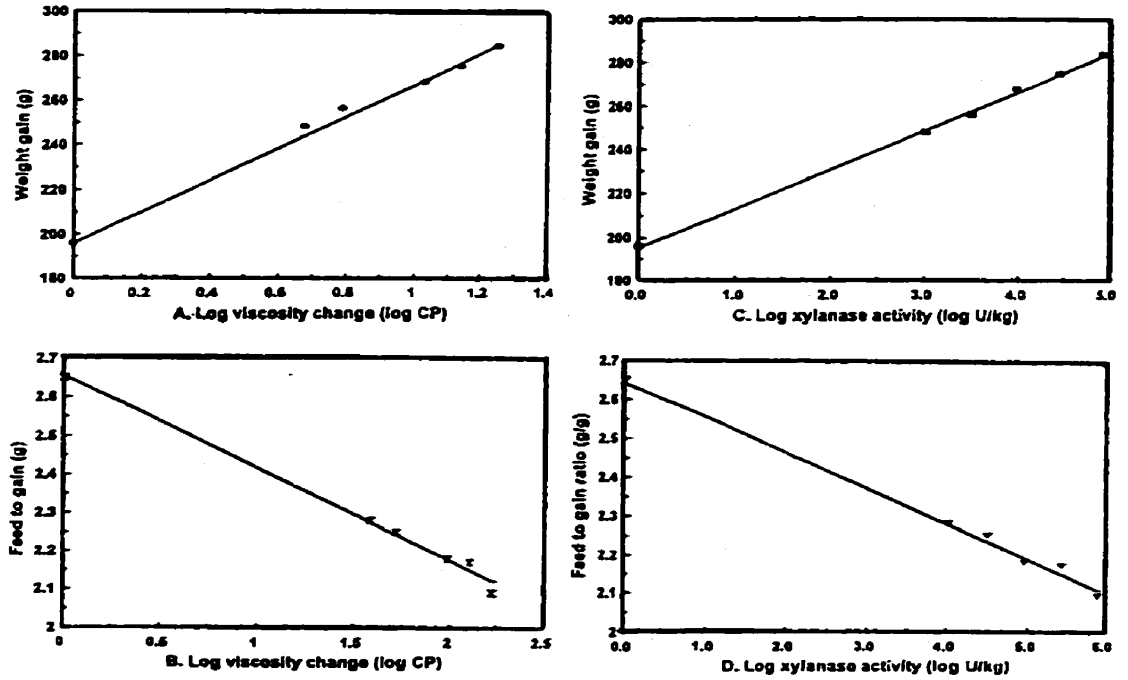

FIGURE 16. Prediction of weight gain (A and C) or the feed to gain ratio (B and D) over a 7-d period for Leghorn chicks fed a rye-based diet containing different concentrations of an enzyme preparation (RM1, Finnfeed International Ltd., Wiltshire, UK) by a log-linear model equation using either log of dietary viscosity change [log centipoise (log CP), A and B] determined by incubation of the diet at 40 C, pH 5.0 for 4 h or log of enzyme activity (log U/kg, C and D) added to the diet as the input of the equation. The linear regression equations were: Y_A=198 + 69.5 log (10 X+1) for weight gain (A) and Y_B=2.65-0.24 log (10^2 X+1) for feed to gain ratio (B) when the input (X) was dietary viscosity change (centipoises, CP), and Y_C=195+18.0 log (10 X+1) for weight gain (C) and Y_D=2.64-0.091 log (10^2 X+1) for feed to gain ratio (D) when the input (X) was enzyme activity added to the diet (U/kg), $r > 0.99$, $P < 0.005$.

predict the response of chickens to added enzyme. The conclusion is also supported that the in vitro assay values as developed in this study can also be utilised in a similar manner. This prediction is important because the amount of enzyme activity in a diet often is not known. Therefore, the newly developed dietary viscosity assay can be used in conjunction with the modified prediction equation to accurately predict the amount of enzyme in the diet and its ability to enhance the performance of chicks. An important feature of this model is that the degree of enhanced performance obtained with a given enzyme product (expressed as log of viscosity or log of amount of enzyme) is a linear function that its B value times the log of its concentration (Zhang et al., 1996, 2000b). As such only a few data points are required to accurately predict response to any given amount of enzyme.

Use of the In Vitro Dietary Viscosity Assay to Evaluate the Efficacy of Enzyme Added to the Diet

We studied the ability of two enzyme preparations (RM1 and NQ) to improve the performance of broiler chicks fed a rye-based diet and to reduce the viscosity of the diet as determined by the in vitro assay. The results showed that both enzyme preparations improved chick performance and decreased the dietary viscosity in a concentration-dependent manner. Two important trends were observed (Table 20). First, the in vitro dietary viscosity assay appeared to be a more sensitive index for evaluating the effect of enzyme response than that obtained from the feeding trial. This conclusion is based on the observation that it was not always possible to distinguish significant differences ($P < 0.05$) in chick performance between successive doses of enzymes because there is a curvilinear effect of enzyme dose on chick

performance. In contrast, changes in viscosity in a well-controlled in vitro assay using the log transformed data is more accurate, especially when the data from the 4 h incubation period were used. This indicates that the in vitro viscosity assay, compared with the results from the feeding trial more accurately predicts enzyme dose. Second, the improvements in chick performance when comparing the response of the two enzyme preparations appeared to be more closely associated with the in vitro dietary viscosity change than the amounts of enzyme added to the diet. For example, the decrease in dietary viscosity with increasing amounts of enzyme added to the diet was larger for NQ ($P < 0.05$, Table 21) than for RM1. This trend was observed even though the xylanase activities added to the diet were slightly lower for NQ than that for RM1 (Table 20). This effect may be attributed to the presence of different amounts of other viscosity-reducing enzymes in the enzyme preparations or to a difference in efficacy of xylanases (i.e., the nature of an enzyme) in the two preparations. These data therefore suggest that the viscosity assay may provide a better index of the efficacy of an enzyme preparation than a standard calorimetric activity assay.

On the basis of these observations it was hypothesized that the in vitro assay, when used with the log-linear model, could be used to accurately predict the efficacy of an enzyme added to a diet. Also, a new approach to evaluate the efficacy of an enzyme added to a diet would be to utilize the B values obtained from the log-linear model equation (Zhang et al., 1996; 2000b, c). The efficacy of the two enzymes (i.e., their B values) were therefore calculated from the model using the dietary viscosity changes as determined by the in vitro assay (Table 20), and the amount of enzyme added to the diet. The results in Table 21 showed that the B value of NQ was significantly greater than that of RM1 (0.265 vs. 0.124, $P < 0.05$) when they

were calculated from log dietary viscosity change as the output of the model and enzyme activity added to the diet as the input. This difference indicates that the enzyme, NQ, more effectively hydrolyzed the viscous substrates in the rye-based diet than RM1. However, there were no significant differences ($P < 0.05$) between the B values of the two enzyme preparations (RM1 vs. NQ) calculated from the chick performance and the enzyme added to the diet (Table 22). The inability of chick performance data to distinguish between the efficacy of the two enzymes in contrast with the in vitro assay may be caused by several factors, including a difference in relative sensitivities or precision of the two assays. In these studies, the relative standard error of means for the in vitro dietary viscosity assay was much lower than that of performance of chickens (Table 20), and as a result it was a more precise indicator of the efficacy of the enzyme than chick performance. The differences in the ability of the two methods to evaluate the efficacy of an enzyme are more clearly indicated in Figure 17. The data show that the response based on the log of dietary viscosity change for NQ (ΔY_{NQ}, log CP) was greater than that of RM1 (ΔY_{RM1}, log CP) when the same increment of enzyme activity was added to the diet (ΔX, log U/kg). In addition, to achieve the same output (Y_i) as log of dietary viscosity change, the amount of enzyme NQ (X_1, log U/kg) required is much lower than that of RM1(X_2, log U/kg). Also, the relative B value of NQ to RM1 was 2.14 when the output of the model was the log of dietary viscosity change at 4 h incubation and the input was the amount of enzyme activity added to the diet, whereas the relative B value was 1.40 when the output was the 1-wk feed to gain ratio and the input was also the amount of enzyme activity added to the diet (Table 21). Also, the results demonstrated that all of the B values obtained from the 2-, 4-, and 8-h incubations and those obtained from the

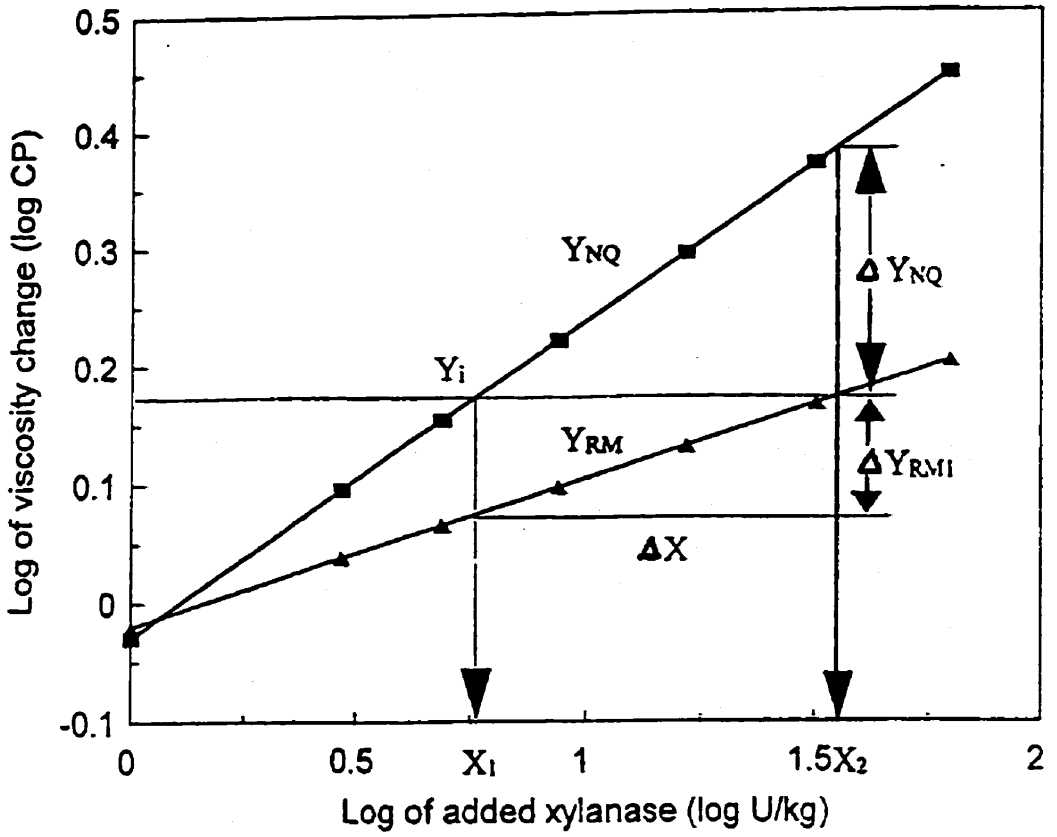

FIGURE 17. Evaluating the efficacy of enzyme using an in vitro dietary viscosity assay in conjunction with a log-linear model, $Y = A + B \log (C X + 1)$. The log of dietary viscosity change [log centipoises (log CP)] as the output of the model was determined by incubation of a rye-based diet containing different amounts of enzyme preparations (RM1, Finnfeeds International Ltd, and NQ, Nutri-Quest) at 40 C, pH 5.0 for 4 h. The linear regression equations in the figure were: Y_{NQ} (log CP) = $-0.020 + 0.124 \log (10^{-2} X + 1)$ for NQ and Y_{RM1} (log CP) = $-0.029 + 0.265 \log (10^{-2} X + 1)$ for RM1 when the input (X) was expressed as enzyme activity added to the diet ($r > 0.99$, $P < 0.005$). The efficacy of enzyme was the slope of the equation, B. The B for NQ = $\Delta Y_{NQ} / \Delta X$, while the B for RM1 = $\Delta Y_{RM1} / \Delta X$, where ΔY_{NQ} or ΔY_{RM1} was an increment of the log of dietary viscosity change (Δlog CP) when log of added xylanase was changed from X_1 to X_2 (ΔX).

1- and 2-wk chick performance data were higher for NQ than for RM1. These data, therefore, suggest that the efficacy of NQ was higher than that of RM1 and that the in vitro viscosity assay may more accurately estimate the potential efficacy of enzymes than the corresponding feeding data.

Assay Evaluation and Further Studies

The results of this study have demonstrated that it is possible to accurately predict the efficacy of an enzyme preparation high in xylanase activity when added to a rye-based diet by the in vitro dietary viscosity assay. An important feature of this assay is that it is carried out in an aqueous suspension and not in a centrifuged aqueous extract of the diet, as carried out in some previously described assays. The disadvantage of the procedures that do not carry out incubations in the presence of the grain matrix is that only a small proportion of the enzyme is extracted from the grain matrix because a high proportion of it is tightly bound to the particulate matter. As a result, the latter assays do not reflect the effective concentration of the enzyme in the digesta. This problem is overcome when the in vitro dietary viscosity assay is carried out in an aqueous suspension of the diet. In addition, the dietary viscosity assay, in contrast with specific enzyme assays, provides a collective measure of all of the enzymes that degrade the viscous compounds in the diet instead of only one single enzyme activity. Furthermore, the in vitro dietary viscosity assay can be used not only to determine the enzyme activity added to the diet, but also the efficacy of the enzyme when used in conjunction with the log-linear model. Therefore, both the qualitative and quantitative features of an enzyme can be accurately evaluated by the in vitro assay.

Although the in vitro dietary viscosity assay, as developed in this study, was highly

correlated with the amount or relative activity of enzyme added to the diet, it cannot be readily used to analyse the activity of enzyme added to diets that have low viscosity, because the amount of substrate (viscous carbohydrate) in the diet can affect the results. The current assay could be further improved by adding high quantities of the appropriate viscous substrates to the diet, especially those with low viscosity, by heat treating part of the diet to eliminate the effects of the enzyme (negative control) and by mixing the heat-treated diet with a known amount of reference enzyme (positive control). Under such conditions, there would be a direct relationship between amount of specific enzyme added to any given diet and its corresponding activity, as estimated from the in vitro viscosity assay. The assay could, therefore, be adapted to diets such as wheat-based diets, in which the concentration of the viscosity factor is much lower. This assay, however, would not provide a basis for predicting chick performance but would predict relative activity of the enzyme. These modifications would increase the sensitivity, uniformity, and therefore, the general applicability of the assay. Further research is required to verify and optimize such an assay, which can therefore predict the amount of enzyme in a diet.

This study demonstrates that it is possible to accurately assess the efficacy of a dietary enzyme and to predict the amount of enzyme in a diet using a new type of in vitro dietary viscosity assay under the conditions of the assay, and that the activity of the added enzyme can be used to predict the ability of the enzyme to enhance performance of chicks fed a diet containing viscous arabinoxylans and probably other viscous compounds. The efficacy of the enzymes proposed in this study can be a powerful tool for evaluating or comparing the effectiveness of different enzyme preparations. However, there may not always be a high

correlation between change in viscosity of certain non-viscous diets and animal performance.

GENERAL DISCUSSIONS

Prediction of Chick Performance and the Log-Linear Model

There has been increasing interest in quantitatively studying the effects of inputs such as different levels of feed enzymes when added to a diet on outputs such as the performance of chickens (Rotter et al., 1989a; Friesen et al., 1991; Bedford and Classen, 1992; Marquardt et al., 1994). The primary objectives of these studies were to estimate the optimal level of the feed enzyme that is required to obtain maximal performance of chickens (Bedford and Classen, 1992; Friesen et al., 1991). However, the experimental designs and analytical procedures used in these studies have only provided descriptive information on the effect of a feed enzyme. Therefore, it has been impossible to establish an accurate quantitative relationship between inputs (enzymes) and outputs (chick performance).

The results from Manuscripts 1 and 2, in contrast to previous studies, demonstrated that the log-linear model was able to accurately predict the performance of chickens fed diets containing different amounts of an enzyme and different proportions of two cereals. The model was tested using data from two dose response studies and those from the literature. High coefficients of correlation were obtained under different experimental conditions [e.g., when enzyme was added to different cereals (rye, wheat, and barley) and a grain legume (lupins), when enzymes were added to different concentrations of two cereals in the diet (wheat and rye), and when these diets were fed to different ages and types of chickens (Leghorn and broiler), and finally when different types of enzymes (β-galactosidase, β-glucanase, and xylanase) were used]. In many of the comparisons, r^2 values of greater than

0.95 were obtained indicating that the prediction equation is accurate. The equation is also simple, because the improvement in performance with enzyme addition is directly related to the logarithmic concentration of the enzyme in the diet. A simple model, as far as we know, has not been used before to predict the response of chickens to a feed enzyme.

The model could be used to determine the response to different amounts of any two dietary components such as different amounts of an enzyme and different proportions of two cereals such as rye and wheat in the diet. Using the data from Bedford and Classen (1992), a multiple regression equation (SAS, 1988) was developed to establish the relationship between chick performance, and the concentration of enzyme and the proportion of rye in the diet ($r^2 = 0.94$, $P < 0.01$).

Efficacy of Feed Enzymes and the Log-Linear Model

Although the major enzymes of many commercial enzyme preparations are labeled as if they all have the same effect on selected cereals (i.e., barley), they often have different pH optimums, substrate preferences, temperature optimums and thermal stabilities because they are derived from different sources primarily including different bacteria (e.g. *Bacillus* sp.) and fungi (e.g., *Aspergillus* and *Trichoderma* sp.). In addition, the reaction conditions where exogenous enzymes act in the gut are determined by the nature of condition in the intestine which are often different from conditions under which the enzymes are assayed (Classen, 1996; van de Mierop and Ghesquiere, 1998). Also, the structure and contents of the target substrates within and between cereals for enzymes are complex and vary in nature. Consequently, the efficacy of most enzyme preparations varies considerably. Currently,

comparison among different enzyme preparations has been often carried out on the basis of the same amount (such as activity) of different enzyme preparations added to a diet. However, it is difficult to correctly evaluate different enzyme preparations based on their activities, since many enzyme preparations are from different sources, contain a different spectrum of enzymes with different catalytic properties. Therefore, the successful development of a method that accurately evaluates the efficacy of a feed enzyme is highly important.

Almquist (1952), in a study of vitamin metabolism in the gut, proved that the slope of a log-linear model was mainly associated with the magnitude of the conversion rate constant for a provitamin to vitamin in the gut. He also demonstrated that the model for evaluating data was extremely useful in many other diverse applications in biology. Feed enzymes, in a similar manner, convert an unavailable nutrient, because of anti-nutritive NSPs in the digesta, to an available nutrient through the degradation of the anti-nutritive NSPs. The magnitude of the conversion should provide a measure of the ability of the enzyme to produce beneficial effects which, as shown in the current thesis, can be reliably predicted using a model equation. This equation is an adaptation of that of Almquist (1952). In this study, it has been shown that the slope of the line (B values) can also be used to evaluate the efficacy of a feed enzyme when it is added to a diet. The B values, in turn, can be used to compare the effects of different enzyme preparations when added to a diet, and to determine the target cereal for a feed enzyme, evaluate the effect of a newly developed enzyme preparation and even the effect of processing on the feed enzyme. Recently, the data from Rotter et al. (1989b) were used to test this hypothesis (Zhang et al., 2000c). In this analysis, the effect of five enzyme

preparations [Cellulase Tv concentrate (CT), Celluclast (CC), Finizym (FZ), Cereflo (CF), and SP249 (SP)] on the performance of Leghorn chicks fed a barley-based diet were compared. The results indicated that the relative B values for the feed to gain ratio were -0.2470, -0.2113, -0.1716, -0.1713, and -0.1386 (g/g per log percentage of enzyme), respectively with CT being much more efficacious than SP. This trend agreed with that of the corresponding overall net improvements ($Y_m - Y_0$) that were obtained. The net improvements in the feed to gain ratios for the five enzyme preparations, CT, CC, FZ, CF, and SP, were -0.71, -0.64, -0.54, -0.58, and -0.45, respectively. The correlation between the B values and the net improvement in feed to gain ratio was high ($r^2 = 0.96$, $P < 0.0041$). This study and other results in Manuscript 4 clearly indicated that the B values accurately reflected the ability of a feed enzyme to improve the performance of chicks. Therefore, it can be considered to be a good measure of the efficacy of an enzyme.

The content and structure of NSPs varies in different cereals (Marquardt, 1997). Therefore, the efficacy of a given feed enzyme on different cereal-based diets, as evaluated by the slope of the log-linear model, should be different. Data from the dose response study of Marquardt et al. (1994) were used to test the hypothesis (Manuscripts 2 and 3). In their study, different concentrations of a feed enzyme (high in both xylanase and β-glucanase activity) were added to corn-, wheat-, barley-, and rye-based diets. The results demonstrated that the effect of the enzyme on chick performance (net improvements) and the B value were highly correlated ($r^2 = 0.90$, $P < 0.001$). These results suggest that the B values of the log-linear equation also can be used to assess the efficacies of a feed enzyme when added to different cereal-based diets.

These data, therefore, suggest that the slope of the log-linear model, the B value, provide a reliable index of the efficacy of an enzyme. This value not only provides a measure of the efficacy of different enzymes when the same diet is used but can also provide a measure of the efficacy of an enzyme when added to different diets or when different amounts of their target substrates are present in the diet. No other procedure, as far as this author knows, has been developed that can accurately estimate the efficacy of an enzyme preparation.

Evaluating and Predicting the Efficacy of Feed Enzymes Based on their Profitability

The log-linear model that was developed in the study can also be used to estimate the maximal economic return when a feed enzyme is added to a diet. Based on the model, a software package, a Multiple Purpose Enzyme Analysis (MPEA), for predicting and evaluating the profitable effect of an enzyme in poultry feeds was developed.

The results from Manuscript 3 demonstrated that the maximum profits obtained for each of the five enzyme preparations, as discussed previously, when added to a barley-based diet were highly correlated with that of the B values for feed to gain ratio ($r^2 = 0.99$, $P < 0.0005$). The results suggested that the B values and the estimated maximal profit provided similar indices for the evaluation of different enzyme preparations when the same price of enzymes was assumed. However, if the price of the enzymes were different, the maximal profit from the use of an enzyme would not only be influenced by its efficacy but also its price.

The results from Manuscript 3 indicated that the maximal profits obtained when an enzyme preparation was added to a barley-, corn-, wheat-, and rye-based diet were \$135.00, \$134.45, \$133.55, and \$123.96 per 1000 birds, respectively. In this analysis, the price of the cereals

was assumed to be the same. However, in this comparison, the correlation, as may be expected, between the B value for performance and the corresponding profitability was not significant ($r^2 = 0.61$, $P = 0.217$). The reason for the low correlation was that the final level of profitability was not only affected by degree of improvement with enzyme but also the final level of performance that was obtained with each cereal. In these studies, the performance was highest for chickens fed barley plus enzyme and lowest for those fed rye plus enzyme even though enzyme addition more dramatically improved chick performance when it was added to the latter compared to the former diet. The results, therefore, suggest that a calculation of the maximum profit is more useful for the feed or enzyme industry in determining which cereal should be used with a given feed enzyme than a calculation of diet that will yield maximal productivity.

The MPEA is also able to determine the optimal amounts of a feed enzyme and a cereal that should be used in a diet to obtain maximal profit. In such an analysis, the pattern of profitability obtained with the addition of different amounts of enzyme to a diet is generally a quadratic rather than the typical hyperbolic pattern obtained when performance is calculated. Therefore, the MPEA, for maximal profits, can be used to calculate: 1) the optimal amount of a given enzyme preparation that should be added to a given cereal-based diet; 2) the optimal amount of a given enzyme preparation that should be added to diets containing different cereals; 3) the optimum amount of each of several different enzymes that should be added to a given diet; 4) and finally the optimal amount of a given enzyme that should be added to a diet that contains varying proportions of two cereals (e.g., rye or barley and wheat or corn). In addition, the MPEA not only can be utilized to indicate optimal combinations of

enzyme and cereal for maximal profit but also the proper combinations to yield a given profit.

As indicated previously, there are many factors that influence the profit obtained when a feed enzyme is added to a diet. They include the amount of enzyme added, the type and amount of cereal, the efficacy of the feed enzyme, and the price of enzyme and cereals used. Once the profit function is established, any variable in the equation can be calculated and analyzed when other factors are fixed. Therefore, as indicated in the study of Manuscript 3, the price that should be paid for an enzyme and a substituted cereal can be determined.

From In Vivo Study to In Vitro Study

Feed enzymes have been evaluated using both in vivo and in vitro procedures. The weaknesses of the in vivo assays are their high variation, low sensitivity, cost, and time requirements. On the other hand, in vitro methods for enzyme assays such as reducing sugar and viscosity assays, the dietary viscosity assay, and digestibility as measured by various incubation procedures, tend to yield low variation and high sensitivities, and are inexpensive and relatively rapid to carry out. Therefore, they are also widely used for the evaluation of a feed enzyme. However, there is generally low correlation between the predicted values obtained from the in vitro methods and the performance of chicks that is obtained when an enzyme is added to the diet. We have recently developed an in vitro dietary enzyme assay that can estimate the amount of a feed enzyme that was added to a rye-based diet and the efficacy of the enzyme. The principle of the assay is to allow the enzyme in the diet to interact with the substrates in a buffered suspension of the diet for an appropriate time period followed by centrifugation of the suspension and the measurement of the residual viscosity of the solution.

As a result, both bound and free exogenous enzymes could react with the soluble, and insoluble NSPs of the diet, similar to the condition in the gut. The results demonstrated that there was a linear relationship ($r > 0.99$, $P < 0.005$) between log of the net viscosity change from the dietary extracts and log of the concentration of enzyme added to the diet.

The results in manuscript 4 also demonstrated that both amount of enzyme added to the diet and the amount of enzyme as determined by the log of the in vitro viscosity assay were linearly related to weight gain and the feed to gain ratio ($r > 0.99$, $P < 0.005$). Therefore, the log of dietary viscosity change not only was able to estimate the enzyme activity added to the diet, but also accurately predicted the performance of chickens when used in conjunction with the modified log-linear model.

In addition, the ability of two enzyme preparations (RM1 and NQ) to improve the performance of broiler chicks fed a rye-based diet and to reduce the viscosity of the diet as determined by the in vitro assay were studied in Manuscript 4. The results showed that both enzyme preparations improved chick performance and decreased the dietary viscosity in a concentration-dependent manner. Therefore, it was hypothesized that the in vitro assay, when used with the log-linear model, could also be used to accurately evaluate the efficacy of an enzyme added to a diet. The efficacy of the two enzymes (i.e., their B values) were then calculated from the model using the dietary viscosity changes as determined by the in vitro assay, and the amount of enzyme added to the diet. The results showed that the B value of NQ was significantly greater than that of RM1 (0.265 vs. 0.124, $P < 0.05$) when they were calculated from log dietary viscosity change as the output of the model and enzyme activity added to the diet as the input. This difference indicates that the enzyme, NQ, more effectively

hydrolyzed the viscous substrates in the rye-based diet than RM1.

In conclusion, a log-linear model has been developed that can be used to accurately predict and evaluate the response of chicks to a feed enzyme in both in vivo and in vitro studies. It is hypothesized that the model and the concept of efficacy proposed in these studies can be readily used to predict and evaluate various responses (i.e., performance, AME, digestibility of feed nutrients, or reducing viscosity of digesta or diets) of different animals (poultry or swine) to different feed enzymes (xylanase, β-glucanase, β-galactosidase, or phytase) as well as other additives or nutrients (i.e., essential amino acids). In addition, a refined level of output (chick performance) and inputs (feed enzyme and substituted cereal), or the most profitable combination of inputs for a specific output can be evaluated by the linear model when only two or three discrete enzyme dose treatments are used. These studies indicate the log-linear model when used in conjunction with a knowledge of nutrition and computer technology can be of considerable assistance to nutritionists in their research activities and business decisions.

CONCLUSIONS AND FURTHER STUDIES

CONCLUSION

1. A simple log-linear model has been developed to predict the response of chickens to a feed enzyme when added to a cereal-based diet. The general model is $Y = A + B \log (C X + 1)$, where Y is the performance value [i.e., weight gain (g) or feed to gain ratio (g/g)], A is the intercept (*y*-axis), B is the slope of the line (change in performance per log unit of enzyme in the diet, C is an adjustment factor, and X is the amount of enzyme when added to a diet. The equation was able to accurately predict the relationships between chick performance and amount of enzyme added to the diet with r^2 values for most comparisons being greater than 0.95 ($P < 0.01$).

2. The slope of the log-linear model (B) provides a measure of the efficacy of a feed enzyme. Therefore, it is a useful index to compare the effect of different feed enzymes on performance of chickens fed a given cereal-based diet or select the target cereal that is most suitable for a given feed enzyme.

3. A Multi-Purpose Enzyme Analyzer has been developed, based on the log-linear model, to evaluate and predict the profitable response of a feed enzyme when added to a diet. The analyzer can be used to compare the profitability of using different enzymes when added to a given cereal-based diet, to select the cereal that will maximize profitability when used with a specific feed enzyme, to determine the optimal amounts of both a feed enzyme and a cereal that should be used to obtain maximal profit, and to even determine the price that should be paid for an enzyme and

a cereal so as to obtain a certain profit.

4. A dietary viscosity assay for enzyme activity, in conjunction with a log-linear model, has been developed to evaluate and predict the response of an enzyme preparation when added to a rye-based diet. There was a high degree of relationship between the log of dietary viscosity change, measured by the assay, and the log of amounts of enzyme added to the diet ($r^2 = 0.99$, $P < 0.005$). Chick performance as affected by enzyme addition could also be accurately predicted using the in vitro viscosity assay. In this assay, dietary viscosity values were transformed to their log values. Also, the assay was able to evaluate the efficacy of different enzyme preparations, especially for those NSP enzymes that hydrolyze the viscous NSP of the diet.

FURTHER POTENTIAL APPLICATION OF THE MODEL

1. The log-linear model when used in conjunction with other methods such as in vivo ileum digestibility or in vitro digestibility of feedstuffs or diets will further improve our ability to evaluate and predict the response of chickens or other animals, such as swine, to a feed enzyme. These new approaches will overcome the limitations of the in vitro dietary viscosity assay. The new approach should be able to evaluate and predict the response obtained when a feed enzyme is added to diets containing low viscosity cereals such as corn and some varieties of wheat. The approaches can also be used to evaluate the effect of any other exogenous feed enzymes such as phytases, proteases, and amylases.

2. The log-linear model will be a useful tool for determining the site of action of

exogenous feed enzymes in the gastrointestinal tract.

3. The log-linear model will also be used to study the interaction among different enzymes, such as xylanases, β-glucanases, proteases, amylases, phytases, and other enzymes, and even other additives, like organic acids, prebiotics, and probiotics. Scientists should, therefore, be able to design improved preparations of enzymes or additives for use in the feed industry.

4. The model itself, in terms of its function for predicting and evaluating feed enzymes, will also be further refined by using multiple inputs, such as the levels of enzymes, cereals, and energy of diets, and availability or digestibility of dietary amino acids; and multiple outputs, such as effects not only on chick performance but also meat quality, microorganism in the gastrointestinal tract, impact on the environment, etc. This multi-factorial model compared to the current model will more closely resemble the practical situation; therefore, it potentially should have a significant impact on the use of feed enzyme technology in the poultry and swine industry.

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