Prediction of the 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 \( Y = A + B \log (CX + 1) \), 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)

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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 several 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 response 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).
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 to 1) 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)\(^3\) and NQ,\(^4\) 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 arabinobioxylan as the substrate. These values were 389 and 778 U/g of enzyme preparation, 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 1. 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\(^3\) from Trichoderma longibrachiatum) in a 4 × 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. (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) × 4 (enzymedoses). 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, 3,276, and 3,349 kcal/kg, 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 1. Bird weight and feed consumption were recorded 4 h after removal of feed at 14 and 21 d of age (Zhang et al. 1996).

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\(^3\)Finnfeeds International Ltd., Wiltshire, UK SN8 1XN.

\(^4\)Nutri-Quest, Chesterfield, MO 63017.
TABLE 1. The relationship between the amount of enzymes (RM1 and NQ) added to a rye-based diet and chick performance (Experiment 1)

<table>
<thead>
<tr>
<th>Item</th>
<th>Enzyme preparation 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enzyme Amount</td>
</tr>
<tr>
<td></td>
<td>Log %</td>
</tr>
<tr>
<td>Activity</td>
<td>U/kg</td>
</tr>
<tr>
<td>Log U/kg</td>
<td></td>
</tr>
<tr>
<td>WG (g)</td>
<td>Week 1</td>
</tr>
<tr>
<td></td>
<td>Yi−Y0</td>
</tr>
<tr>
<td></td>
<td>Week 2</td>
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<tr>
<td></td>
<td>Yi−Y0</td>
</tr>
<tr>
<td></td>
<td>2 wk</td>
</tr>
<tr>
<td></td>
<td>Yi−Y0</td>
</tr>
<tr>
<td></td>
<td>F/G (g/g)</td>
</tr>
<tr>
<td></td>
<td>Yi−Y0</td>
</tr>
<tr>
<td></td>
<td>Week 2</td>
</tr>
<tr>
<td></td>
<td>Yi−Y0</td>
</tr>
<tr>
<td></td>
<td>2 wk</td>
</tr>
<tr>
<td></td>
<td>Yi−Y0</td>
</tr>
</tbody>
</table>

1The raw data are from Zhang et al. (1996).
2Enzyme preparations, RM1 (Finnfeed 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).
3Yi and Y0 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. Yi−Y0 is net improvement in performance relative to the diet without enzyme addition when a certain concentration of enzyme is added to the diet.
4WG = weight gain; F/G = feed to gain ratio.

Analyses of Data

**Development of a Computer Program.** The outline of the program that was developed to calculate the parameters for the modified log-linear model was written in QuickBASIC 5 (Figure 1). 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 1010), and the lowest value was the starting value. A least squares regression for each C was calculated 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 (r²) and the lowest standard error of the estimated mean (S_y), as determined by the method of least squares. The F-value was also calculated to test the hypothesis of the regression model. The same r², 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 (%).

5Microsoft Canada, Inc., 6300 Northwest Drive, Mississauga, Ontario, L4V 1J7, Canada.
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 $Y = A + B \log (CX + 1)$, 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 1 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 × 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 2). 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 chick performance 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:

$$Y = A + B \log X \quad \text{(0)}$$

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 ($\varepsilon$) was selected that was very small and close to zero (Equation 1).

$$Y = A + B \log (X + \varepsilon) \quad \text{(1)}$$

Zhang et al. (1996) reported that the $\varepsilon$ value was constant, as it was not affected by enzyme concentration for a given diet. Therefore, it was possible to select an appropriate $\varepsilon$ 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).
were utilized to overcome these weaknesses. The following equations may not affect the slope $B$ of Equation 1 have been 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 $\epsilon$ 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 ($\epsilon$) is used. As such $A = Y - B \log \epsilon$ may not yield an accurate estimate of chick performance for diets that do not contain added enzyme. In turn, the selected $\epsilon$ may also affect the slope $B$ of Equation 1 [B = $(Y - A)/\log (X + \epsilon)$]. These are weaknesses of the previous model developed by Zhang et al. (1996). The following equations were utilized to overcome these weaknesses.

### 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 ($\epsilon$) is used. As such $A = Y - B \log \epsilon$ may not yield an accurate estimate of chick performance for diets that do not contain added enzyme. In turn, the selected $\epsilon$ may also affect the slope $B$ of Equation 1 [B = $(Y - A)/\log (X + \epsilon)$]. These are weaknesses of the previous model developed by Zhang et al. (1996). The following equations were utilized to overcome these weaknesses.

#### Modification of Equation 1

As discussed before, the introduction of an $\epsilon$ value into Equation 1 not only decreases the accuracy of certain parameters, such as $A$, but its value is difficult to calculate; therefore, an arbitrary $\epsilon$ value must be selected (Zhang et al., 1996). However, a different approach as outlined below can be used to solve this problem. In this approach $\epsilon$, in Equation 1, is assigned a value of 1 ($\epsilon = 1$), and $X$ is amplified several-fold by use of a constant ($C$). Therefore, the newly modified equation is as follows:

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

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

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

### Table 2. Evaluation of the performance of Single Comb 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) [2]

<table>
<thead>
<tr>
<th>Item</th>
<th>RM1</th>
<th>NQ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(g/kg)</td>
<td>(%)</td>
</tr>
<tr>
<td>Item</td>
<td>Week 1</td>
<td>Week 2</td>
</tr>
<tr>
<td>A</td>
<td>195</td>
<td>334</td>
</tr>
<tr>
<td>$Y_0$</td>
<td>187</td>
<td>329</td>
</tr>
<tr>
<td>B</td>
<td>196</td>
<td>345</td>
</tr>
<tr>
<td>$Y_m - Y_0$</td>
<td>88</td>
<td>60</td>
</tr>
<tr>
<td>$B/A \times 10^{-2}$</td>
<td>9.2</td>
<td>3.4</td>
</tr>
<tr>
<td>$(Y_m - Y_0)/Y_0$</td>
<td>0.45</td>
<td>0.17</td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.99</td>
<td>0.64</td>
</tr>
<tr>
<td>$(\epsilon^2)$</td>
<td>0.96</td>
<td>0.74</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

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

2RM1 enzyme preparation from Finnfeed International, Ltd., Wiltshire, UK SN8 1XN; NQ enzyme preparation 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 amount of enzyme added to the diet and its effect on chick performance; $C$ is an adjusted factor used to correct the $X$ value when enzyme is not added to the diet. $A$ values are the $A$ values as estimated from Equation 1, $Y = A + B \log (CX + \epsilon)$ that were originally reported by Zhang et al. (1996). $Y_m$ and $Y_0$ are the respective observed performance values of chicks [weight gain (g) or feed to gain ratio (g/g)] for the indicated periods $\epsilon$ is the adjusted factor used to correct the $X$ value when enzyme is not added to the diet. $X$ in Equations 1 and 2 (Experiment 1) $A$ and $B$, values are the $A$ values as estimated from Equation 1.

4$WG = weight gain; F/G = feed to gain ratio.
Because $C \times 0 = 0$, and $B \log 1 = 0$, then

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

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 $\varepsilon$ 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 $\varepsilon$ (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 1). In this program, $A$, $B$, and $C$ values were calculated with Equation 1. Similar results were obtained in Tables 1 and 2. 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 $\varepsilon$ 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 1), similar activities of two enzyme preparations (RM1 and NQ) were added to the rye-based diet. The net improvement in performance ($Y_1 - Y_0$) with different dietary concentrations of enzyme (i) was used to calculate the corresponding B values (Table 2). The respective B values of NQ compared to RM1 in the first week for weight gain and the feed/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.

### Table 3. 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)

<table>
<thead>
<tr>
<th>Item $^2$</th>
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<th>20</th>
<th>40</th>
<th>60</th>
<th>20</th>
<th>40</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y_0$ ($\text{g}$)</td>
<td>232</td>
<td>399</td>
<td>359</td>
<td>306</td>
<td>232</td>
<td>399</td>
<td>359</td>
</tr>
<tr>
<td>$B$</td>
<td>1.64</td>
<td>1.73</td>
<td>1.83</td>
<td>2.28</td>
<td>1.64</td>
<td>1.73</td>
<td>1.83</td>
</tr>
<tr>
<td>$C$</td>
<td>1.83</td>
<td>1.83</td>
<td>1.83</td>
<td>1.83</td>
<td>1.83</td>
<td>1.83</td>
<td>1.83</td>
</tr>
</tbody>
</table>

$^1$Data were calculated from that reported by Bedford and Classen (1992).

$^2$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. Parameter C is the amplified factor. The bracketed A1 values, in contrast to A, were calculated with Equation 1. $r^2$ and $r_1^2$ values are the correlation coefficients between the observed experimental values and the predicted values when Equation 1 ($r_1^2$) or Equation 2 ($r^2$) was used.

$^3$Values refer to percentage rye gain in diet. Wheat was the other cereal used. Total cereal in diet was 60%.

$^4$WG = weight gain; F/G = feed to gain ratio.
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 2). 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 2 were 11.3 vs. 9.26 g per 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 3). Bedford and Clausen (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, and 0% 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., arabininoxylan) in the diet. Again as shown in Table 3, 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 \(\beta\)-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 4 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 \(-1.67, 0.081, 2.26,\) and \(8.76 \text{ g/log}\) 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 \((Y_m - Y_0)/\text{amount of enzyme}\) 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 \((Y_m - Y_0)/\text{amount of enzyme value}\) 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 \((Y_m - Y_0)/\text{amount of enzyme}\).

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:

\[
B = (Y - A)/\log (C X + 1) \tag{5}
\]

**Relative B value (B/A) and the Relative Efficacy of an Enzyme.** The relative improvement of chick performance \([(Y_1 - Y_0)/Y_0 \text{ or } (Y_2 - Y_0)/Y_0]\) for RM1 and NQ is greater in the first week than in the second week for the value for the two periods being approximately the mean of the two values between them (Table 1 and 2). In contrast, the B values in Table 2 are not approximate and are additive for Weeks 1 and 2, which is 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:

\[
B/A = [(Y - A)/A]/\log (C X + 1) \tag{6}
\]

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 2, 3, and 4.

**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 then being constant as expected. The reason for this result may be that the relative efficacy of an enzyme on 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 \(\beta\)-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 Chot, 1991; Bedford, 1997). The addition of enzyme preparations containing xylanase and \(\beta\)-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 that collectively can be assessed from its B value.

**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 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 is possible to use these values to efficiently 1) establish 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, \( k_{cat} \), \( k_{cat}/K_{m} \), 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 10³ for weight gain or 10⁴ for feed to gain ratio as shown in Table 2. In addition, the ratio between the two terms expressed (CX and 1) as units of NQ was approximately 10³. Therefore, the addition of 1 to the CX number is relatively insignificant. Similar large ratios were also used in Tables 3 and 4. 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 2 to 4). 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 2, 3, and 4). For example, in Table 2 the \( r^2 \) values for the feed to gain ratio with NQ enzyme for Week 1, Week 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. (2000). 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 feedstuff. 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.

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