

Use of Simultaneous Curve Fitting and a Four-Parameter Logistic Model to Evaluate the Nutritional Quality of Protein Sources at Growth Rates of Rats from Maintenance to Maximum Gain

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ABSTRACT A four-parameter logistic model was used to describe the dose-response relationship of rats fed diets containing 12 levels of casein, peanut meal or wheat gluten. The model was capable of accurately describing the entire response curve of rats fed diets containing each of the three protein sources. Incorporation of a technique known as parameter sharing into the curve-fitting process facilitated convergence of the parameter estimates for b (the response of rats fed a protein-free diet) and R_{\max} (maximum response) for all curves when compared with the values observed experimentally. Parameter sharing also provided a method by which the curves could be differentiated on a statistical basis. These data indicate that the relative value of a protein source is dependent on the concentration of the protein in the diet. The application of nonlinear models combined with parameter sharing provides a technique by which protein values can be evaluated at levels of animal response from maintenance to maximum growth. *J. Nutr.* 117: 1681–1688, 1987.

INDEXING KEY WORDS:

• protein quality • logistic model • parameter sharing • nonlinear models • casein • peanut meal • wheat gluten • rat

A variety of biological methods are currently used to evaluate the nutritional quality of proteins or protein mixtures (1). Most of these methods evaluate the relative quality of a protein or protein mixture at a previously determined dietary protein concentration and therefore must assume this value is constant across a range of dietary concentrations. A variation of these methods is the slope-ratio technique in which the test animal is fed protein or protein mixtures at several dietary concentrations and the value of the protein or protein mixture is determined by the slope of a linear regression equation (2). It is now recognized that most

nutrient (dose) response relationships are not linear and thus single-point or linear assays cannot accurately determine the value of a protein or protein mixture across a wide range of dietary protein concentrations (3–5).

A number of nonlinear models have been used in an effort to describe the entire dose-response relationship of animals fed diets containing a wide range of dietary protein concentrations (6–8). Although these models are often useful in accurately describing the entire dose-response curve, they have been criticized for lacking the ability to statistically differentiate between protein sources, which can easily be distinguished using linear methods (5, 9).

In this paper we apply a technique first described by Waud (10, 11) to evaluate statistically the dose-response relationship of young growing rats fed a series of diets containing one of three different protein sources of widely varying qualities. The technique, known as parameter sharing, allows the simultaneous analysis of a series of dose-response curves (12). The model used to analyze these data will be a modification of the four-parameter logistic model of Richards (13).

METHODS

The model. The model used is the four-parameter logistic model (8, 13) shown below:

$$\text{Response} = \frac{R_{\max} + R_{\min} \cdot c \cdot d^I}{1 + c \cdot d^I} \quad (1)$$

The parameters are defined as follows (8): I , nutrient intake; c , parameter related to initial body size that determines growth rate; d , growth factor associated with

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nutrient quality; R_{\min} , theoretical response at an intake of negative infinity (in grams); R_{\max} , maximum response (in grams).

This model can be modified by factoring the parameter R_{\min} out of the equation and replacing it with another parameter, b , which can be defined as the response at zero nutrient intake (the y-intercept). From the model, parameter b can be defined as:

$$b = \frac{R_{\min} + R_{\max} \cdot c}{1 + c} \quad (2)$$

Parameter R_{\min} can then be expressed as a function of b as follows:

$$R_{\min} = \frac{b \cdot (1 + c) - R_{\max}}{c} \quad (3)$$

Substituting this into the original equation, the new model then becomes

$$\text{Response} = \frac{R_{\max} + [b \cdot (1 + c) - R_{\max}] \cdot d^I}{1 + c \cdot d^I} \quad (4)$$

This change increases the utility of the model since parameter b is a point that represents a measurable value (in this application it is the response of animals fed a protein-free diet), whereas R_{\min} represents only a theoretical point on the curve. In the analysis of a number of dose-response curves, it was found that parameter R_{\min} was highly correlated with parameter c (14). In contrast, parameter b was not highly correlated with any of the other parameters, suggesting that it is an independent parameter and a necessary component of the equation (15).

A consequence of this restructuring of the logistic equation is that the parameters c and d can be redefined so that when curves differ with respect to either of these parameters the implications of these differences can be more easily explained. Table 1 and Fig. 1 show the effect of varying the parameters c and d on the response curve when the parameters b and R_{\max} were held constant.

In this example the relative value is used to determine the effects of various combinations of values for parameters c and d on the dose-response curve. The relative value is calculated by dividing the dose required to achieve a specific response from the control curve (i.e., curve 4) by the dose required to achieve the identical response from the test curve. For example, curve 1 requires a dose of 6.12 to reach 95% of the predicted R_{\max} , while curve 4 requires a dose of 18.37. The relative value of curve 1 at this response is $18.37/6.12$ or approximately 300% that of curve 1. The roles of parameters c and d in the logistic equation can be more easily seen in the following discussion.

Equations that differ only in the value for parameter d (Table 1, curves 2, 4 and 6) result in a shifting of the curve from curve 4 (Fig. 1) to the left (lower d , curve 2) or right (higher d , curve 6) with no change in the shape of the curve (note the constant relative value for

TABLE 1

Predicted dose required to achieve 0, 30, 60, or 95% of the maximum response using the logistic equation¹

Curve	Percent of R_{\max}			
	0	30	60	95
1 $d = 0.60; c = 0.1$				
Dose	0.08	0.84	1.98	6.12
Relative value	603.28	481.11	396.53	300.36
2 $d = 0.60; c = 2.0$				
Dose	0.21	1.76	3.44	8.03
Relative value	228.92	228.92	228.92	228.92
3 $d = 0.80; c = 0.1$				
Dose	0.19	1.91	3.44	8.03
Relative value	263.53	210.16	173.21	131.21
4 $d = 0.80; c = 2.0$				
Dose	0.49	4.02	7.87	18.37
Relative value	100.00	100.00	100.00	100.00
5 $d = 0.95; c = 0.1$				
Dose	0.81	8.32	19.76	60.91
Relative value	60.58	48.31	39.82	30.16
6 $d = 0.95; c = 2.0$				
Dose	2.13	17.47	34.23	79.92
Relative value	22.99	22.99	22.99	22.99

¹Values for parameters c and d are as indicated. The values for parameters b and R_{\max} were fixed at -0.25 and 6.5 , respectively. These values simulate growth of rats fed diets containing no protein or adequate protein. The curve with $d = 0.80$ and $c = 2.0$ was used as the standard to calculate relative values.

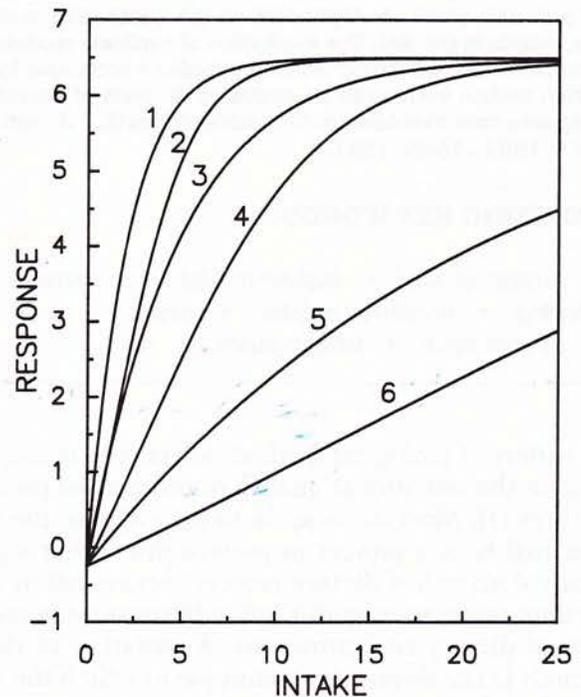


FIGURE 1 Effect of varying parameters c and d on the shape of the dose-response curve using the four-parameter logistic model. The values for parameters b and R_{\max} were fixed at -0.25 and 6.50 , respectively. Curve 1, $d = 0.60$, $c = 0.1$; curve 2, $d = 0.60$, $c = 2.0$; curve 3, $d = 0.80$, $c = 0.1$; curve 4, $d = 0.80$, $c = 2.0$; curve 5, $d = 0.95$, $c = 0.1$; curve 6, $d = 0.95$, $c = 2.0$.

curves 2 and 6 in relation to curve 4; Table 1). These curves have the same shape but differ with respect to their scaling in relation to the x-axis. From this it is apparent that parameter d functions as a "scaling parameter" and has no effect on the actual shape of the response curve.

Thus, in relation to the use of this technique to evaluate the quality of proteins, significant differences solely in parameter d are an expression of different relative values of the proteins that will be maintained over the entire response range.

Equations that differ only in the value for parameter c result in a change in the shape of the curve as evidenced by the constantly changing relative values of curves 1, 3 and 5 in relation to curves 2, 4, and 6 (Table 1). Again in relation to the use of this technique to estimate protein quality, equations with a common value for d but with lower values for parameter c show constantly decreasing relative values for one protein or a series of proteins in relation to a standard, while equations with higher values for parameter c show constantly increasing relative values. From this it appears that parameter c functions as a "shaping parameter." Finally, the change in the relative qualities of proteins with dose is greater when there is a significant difference in both parameters d and c than when the difference is only in parameter c .

The final equation and the definition of the parameters are as follows:

$$\text{Response} = \frac{R_{\max} + [b \cdot (1 + c) - R_{\max}] \cdot d^I}{1 + c \cdot d^I}$$

where I is the nutrient intake, c the shaping factor, d the scaling factor, b the response at zero intake (in grams) and R_{\max} the maximum response (in grams).

The final fit of the model to the data is a function of the interaction of all four parameters. At low levels of nutrient intake, parameter b is the major factor affecting the predicted response. This is demonstrated in Fig. 1. Regardless of the value of parameter c or d , all curves converge at a common value at low levels of intake. This can be seen from the equation above as well because as the value of I approaches zero, d becomes 1 and the equation reduces to $B/1$. As the response approaches the plateau, R_{\max} becomes the most important parameter affecting the dose-response relationship. This is seen in Fig. 1 because curves 1-4 converge on a common value even though different levels of intake are required. This is also seen in the equation because as I approaches infinity, d approaches zero and the equation reduces to $R_{\max}/1$. Between these two extremes the parameters c and d are most responsible for the response at a given intake. Lower values for parameters c and d increase the slope of the curve, while higher values for c and d diminish the slope. Interestingly, the mathematical relationship between parameters c and d is such that an increase or decrease in d cannot be offset by an increase or decrease in c of the

same magnitude. In the logistic equation parameter d is always raised to the power of the intake (d^I) while parameter c is not mathematically bound to any other variable.

Statistical analysis. Waud (10, 11) first described a technique by which a series of dose-response curves could be fitted simultaneously. Later, DeLean et al. (12) described a computer program written for the simultaneous analysis of families of dose-response curves commonly encountered in pharmacological studies. This program allows the user to obtain more information from a series of related curves than could be obtained by fitting each curve separately. This is accomplished by placing constraints on the model and statistically evaluating the effects of the constraints using the fit of the unconstrained model for comparison.

Parameter sharing is one type of constraint whereby two or more curves can be forced to share a common value for one or more parameters (12). By using this technique it is possible to statistically determine the need for independent parameters for different dose-response curves. Curves that should have a common value for one or more parameters can be forced to share an identical value (i.e., maximum growth). The final estimate of the value for the parameter is then based on the combined data from the individual curves.

A second type of constraint involves assigning a parameter a fixed value, thereby reducing the number of fitted parameters by one. When the value of a parameter can be experimentally determined, assigning it a fixed value has been found to be useful in helping to obtain an adequate fit of the model to the experimental data (5, 16). This option is available in many commercial nonlinear regression routines.

Two criteria are available for evaluating the effect of a constraint on the fit of the model to the data. The first is the principle of the extra sum of squares (12). Any constraint results in an increase in the sum of squares since the model is not free to find the best fit to the experimental data. Since the degrees of freedom are calculated by subtracting the total number of parameters fitted from the total number of data points, any type of constraint also increases the error degrees of freedom associated with the model. Fixing a parameter effectively removes it from the model, thereby increasing the error degrees of freedom by one. The increase in the degrees of freedom when parameter sharing is used can be calculated by subtracting the number of parameters shared from the number of curves sharing those parameters.

Designating the sum of squares and the error degrees of freedom of the unconstrained fit as SS_0 and DF_0 , respectively, and that of the constrained fit as SS_1 and DF_1 , then the F -ratio for analyzing the extra sum of squares is calculated as shown (12):

$$F = \frac{(SS_1 - SS_0)/(DF_1 - DF_0)}{(SS_1 + SS_0)/(DF_1 + DF_0)}$$

If the gain in the number of error degrees of freedom is offset by the increase in the sum of squares, the F -ratio will be small, indicating an appropriate fit. A large difference between the initial fit and the constrained fit will result in a large F -ratio, indicating a decrease in the goodness of fit.

The second criterion for judging the validity of a constraint is based on the distribution of the residuals around the fitted regression line. Through the use of a run test the distribution of the residuals can be evaluated non-parametrically for nonrandomness (12, 15). A large number of residual runs indicates a random distribution of the residuals and a good fit of the model to the data. A small number of residual runs is indicative of a nonrandom distribution of the residuals, which can be interpreted as a systematic deviation of the data from the model. This indicates that the model is no longer appropriate.

The program used in these analyses was written in extended BASIC for the Sperry Univac 1100 at the University of Wisconsin-Madison Academic Computing Center and designed to be used interactively. The program is based on a similar program written by DeLean et al. (12). A number of modifications have been made to increase the program's usefulness. These include 1) the addition of a sort routine to arrange the data into ascending order, 2) the addition of a routine to plot the residuals of each fit in order to more fully evaluate the adequacy of the fit of the model to the data and 3) automatic calculation of intakes, slopes and relative values at points along the response curve.

Experimental procedures. The data used in this analysis were from a 4-wk experiment reported by Phillips (5) in which rats were fed diets containing graded levels of protein from one of three sources, casein, peanut meal or wheat gluten. For each protein source, 12 isocaloric diets were formulated containing from 2 to 31% crude protein. Six weanling male rats were assigned to each of the 36 treatments. In addition, 12 animals were fed a protein-free diet. Food and water were provided ad libitum. Food intake and weight gain were measured weekly. Twelve animals were killed before the start of the experiment to determine the values for body nitrogen at the start of the experiment. At the end of the 4-wk period the animals fed the experimental diets were killed and the carcasses analyzed for nitrogen. In this article, nitrogen retention (final body nitrogen minus initial body nitrogen) was used as the dependent variable and nitrogen intake as the independent variable. A more detailed explanation of the experimental protocol can be found in the papers of Phillips (5, 17).

RESULTS

The results of fitting the four-parameter logistic model to the data, with and without the sharing of one or more parameters, are shown in **Table 2**. For the first

fit, no constraints were placed on the model, meaning that the three curves were fit independently. Since all curves should have a common y -intercept, for the second fit the three curves were constrained to share a common value for parameter b because this parameter represents the response of animals at zero protein intake. As expected, this constraint had no significant effect on the total sum of squares, the sum of squares or the residual run pattern of any of the three individual curves. The third fit placed two constraints on the model, a common value for parameters b and R_{\max} (this parameter represents the maximum gain), since both the gain at zero intake and the maximum gain should be independent of the protein source. When these two constraints were placed on the model, no significant effects on either the sum of squares or the residual run patterns were observed. The fourth fit builds on the previous one and places an additional constraint on the model, that of a common value for parameter c . While this additional constraint had no effect on the overall sum of squares as evidenced by the extra sum of squares test, it significantly altered the distribution of the residuals as evidenced by the significant ($P < 0.05$) run test for curve 1 (casein). This indicated that this constraint resulted in a decrease in the goodness of fit of the model to the data.

When all curves were constrained to share a common value for parameters b and R_{\max} (fit 3), inspection of the data revealed that curve 3 (wheat gluten) had a value for parameter c quite different from that of curve 1 (casein) or curve 2 (peanut meal). Therefore, for the fifth fit, all curves shared a common value for parameters b and R_{\max} while only curves 1 and 2 shared a common value for parameter c . This allowed an independent estimation of parameter c for curve 3. Inspection of these results indicated a suitable fit of the model to the data as evidenced by both the extra sum of squares test ($F = 0.02$; $P = 1.00$) and the residual run test. Again building on the previous fit, the sixth fit forced all curves to share a common value for parameters b , R_{\max} and d , while only curves 1 and 2 shared a common value for parameter c . In this analysis the model for curves 1 and 2 was identical. These constraints resulted in a poor fit of the model to the data as evidenced by the result of the extra sum of squares test ($F = 3.57$; $P = 0.00$) or the residual run test (**Table 2**).

From these analyses the fifth fit was selected as the most appropriate model. In this fit all curves were able to share a common value for parameters b and R_{\max} , and curves 1 and 2 could also share a common value for parameter c . The effects of parameter sharing on the fit of the model to the three sets of data are examined in more detail in **Table 3**. For all curves it appeared that parameter sharing had little effect on the sum of squares or the number of residual runs. Parameter sharing had little effect on any of the parameter estimates for the curves describing the response of rats fed diets containing casein or peanut meal. There were

TABLE 2
Final parameter estimates, sum of squares and statistical goodness-of-fit tests for sequential fitting of the logistic model to the response of rats fed diets containing three different protein sources

Fit no.	Parameters shared	Curve no. ¹	Parameter estimates				Sum of squares	Degrees of freedom	F-ratio (probability)	Residuals		Number of runs (P-value)
			b	c	d	R _{max}				Positive	Negative	
1	None	1	-0.267	4.800	0.706	6.413	15.155	78.00		39	43	44 (P > 0.05)
		2	-0.276	3.048	0.842	6.554	7.501	77.00		41	40	43 (P > 0.05)
		3	-0.251	-0.022	0.989	12.936	1.827	77.00		34	47	30 (P < 0.05)
		Total					24.483	232.00		114	130	
2	b ₁ , b ₂ , b ₃	1	-0.263	4.841	0.705	6.413	15.155	78.67		38	44	44 (P > 0.05)
		2	-0.263	3.131	0.841	6.544	7.504	77.67		39	42	41 (P < 0.05)
		3	-0.263	-0.294	0.992	13.842	1.829	77.67		35	46	30 (P < 0.05)
		Total					24.484	234.00	0.02 (P = 0.98)	112	132	
3	b ₁ , b ₂ , b ₃ R _{max1} R _{max2} R _{max3}	1	-0.260	4.778	0.707	6.428	15.160	79.33		38	44	44 (P > 0.05)
		2	-0.260	3.433	0.833	6.428	7.533	78.33		40	41	40 (P > 0.05)
		3	-0.260	0.636	0.963	6.428	1.830	78.33		35	46	30 (P < 0.05)
		Total					24.522	236.00	0.00 (P = 1.00)	113	131	
4	b ₁ , b ₂ , b ₃ c ₁ , c ₂ , c ₃ R _{max1} R _{max2} R _{max3}	1	-0.224	3.681	0.729	6.467	15.448	80.00		36	46	38 (P < 0.05)
		2	-0.224	3.681	0.832	6.467	7.559	79.00		37	44	39 (P > 0.05)
		3	-0.224	3.681	0.925	6.467	2.368	79.00		49	32	27 (P < 0.05)
		Total					25.376	238.00	0.04 (P = 1.00)	122	122	
5	b ₁ , b ₂ , b ₃ , c ₁ , c ₂ R _{max1} , R _{max2} R _{max3}	1	-0.261	3.863	0.724	6.445	15.279	79.83		37	45	42 (P > 0.05)
		2	-0.261	3.863	0.828	6.445	7.598	78.83		42	39	43 (P > 0.05)
		3	-0.261	0.619	0.963	6.445	1.831	78.33		35	46	30 (P < 0.05)
		Total					24.708	237.00	0.02 (P = 1.00)	114	130	
6	b ₁ , b ₂ , b ₃ , c ₁ , c ₂ d ₁ , d ₂ , d ₃ , R _{max1} R _{max2} R _{max3}	1	-0.240	0.764	0.874	6.998	48.218	80.50		57	25	18 (P < 0.05)
		2	-0.240	0.764	0.874	6.998	37.622	79.50		5	76	9 (P < 0.01)
		3	-0.240	13.289	0.874	6.998	6.411	79.00		61	20	15 (P < 0.05)
		Total					92.252	239.00	3.57 (P = 0.00)	123	121	

¹Curve 1, casein; curve 2, peanut meal; curve 3, wheat gluten.

only minor changes in the values for parameters *b*, *c* and *d* for the curve describing the response of rats fed diets containing wheat gluten. In contrast, the value for *R*_{max} when all possible parameters were shared (6.445 g) was only half that of the initial estimate (12.936 g) when no parameters were shared. When the three curves were forced to share a common value for *R*_{max}, the estimate of *R*_{max} for the model describing the response of rats fed diets containing wheat gluten became similar to that of the other two models when parameters were not shared (6.445 vs. 6.413 and 6.554). This example also shows that when parameters are shared the resulting value is not merely an average of the three values obtained independently but rather is the result of each curve supplying a variable amount of information to the final parameter estimate.

Another effect of parameter sharing was the effect on the standard error estimates of the parameters. While these standard errors are not the same as the confidence limits seen in linear regressions, they do give an indi-

cation of the precision of the parameter estimate (15). Again the most dramatic example was seen for the model describing the response of rats fed diets containing wheat gluten (Table 3). When no parameters were shared, the standard error for parameters *c* and *R*_{max} was 1900- and 3.6-fold that of the actual value of the parameter. By sharing parameters, the standard errors were reduced to less than 2% of the initial values, indicating a great increase in the precision of the parameter estimates.

The effect of parameter sharing on the fit of the model to the data can also be seen in Fig. 2. Note that in all three cases the lines for the initial fit (without parameter sharing) and the fifth fit (with parameter sharing) were nearly indistinguishable.

DISCUSSION

In this paper we describe a technique that allows simultaneous fitting of a series of dose-response curves

TABLE 3

Effect of average parameter estimates without and with parameters shared on the fit of the logistic model to the response of rats fed casein or peanut or wheat gluten as the sole source of dietary protein¹

Parameters	Protein source	Parameter estimates				Sum of squares	Runs
		<i>b</i>	<i>c</i>	<i>d</i>	<i>R</i> _{max}		
Not shared	Casein						
	Mean	-0.267	4.800	0.706	6.413	15.155	44
	SEM	±0.085	±1.215	±0.021	±0.087		
Shared	Casein						
	Mean	-0.261	3.863	0.724	6.445	15.279	42
	SEM	±0.045	±0.622	±0.014	±0.079		
Not shared	Peanut meal						
	Mean	-0.276	3.048	0.842	6.554	7.501	43
	SEM	±0.078	±0.859	±0.019	±0.236		
Shared	Peanut meal						
	Mean	-0.261	3.863	0.828	6.445	7.598	43
	SEM	±0.045	±0.622	±0.009	±0.079		
Not shared	Wheat gluten						
	Mean	-0.251	-0.022	0.989	12.936	1.827	30
	SEM	±0.081	±42.185	±0.867	±47.224		
Shared	Wheat gluten						
	Mean	-0.261	0.619	0.963	6.445	1.831	30
	SEM	±0.045	±0.948	±0.017	±0.079		

¹The fit used is number 5 from Table 2.

using a four-parameter logistic model to describe the data. Other models (6, 18) were tested using this technique, but the four-parameter logistic model described here consistently provided the best fit to the data as judged by both the lowest sum of squares and the distribution of the residuals around the regression line. In addition, this model provided the closest estimates of both the maximum response and the response of animals fed a protein-free diet.

The results presented in this report clearly demonstrate that one advantage of using parameter sharing in the analysis of nutrient (dose) response curves is the ability to differentiate statistically between the parameter estimates for the three curves (5, 9). When the three curves were fit independently, the parameter estimates for the model describing the response of rats fed diets containing wheat gluten were not statistically different (using the standard errors as approximate confidence limits) from those describing the response of rats fed diets containing either casein or peanut meal. In contrast, when parameter sharing was used, the models describing the response of rats fed diets containing either casein or peanut meal were significantly different from the model describing the response of rats fed diets containing wheat gluten. These differences were the result of different values for the parameters *c* and *d*.

Another advantage gained by the simultaneous analysis of dose-response curves is that improved estimates for all parameters become possible. Since the fit of the model to the data is the result of an interaction of all the parameters, a more accurate estimate of each parameter is possible, which facilitates the estimates of

the other parameters (4, 16). Many protein sources are of such low quality that the maximum response may not be possible even at high dietary protein concentrations (8). In this experiment rats fed diets containing

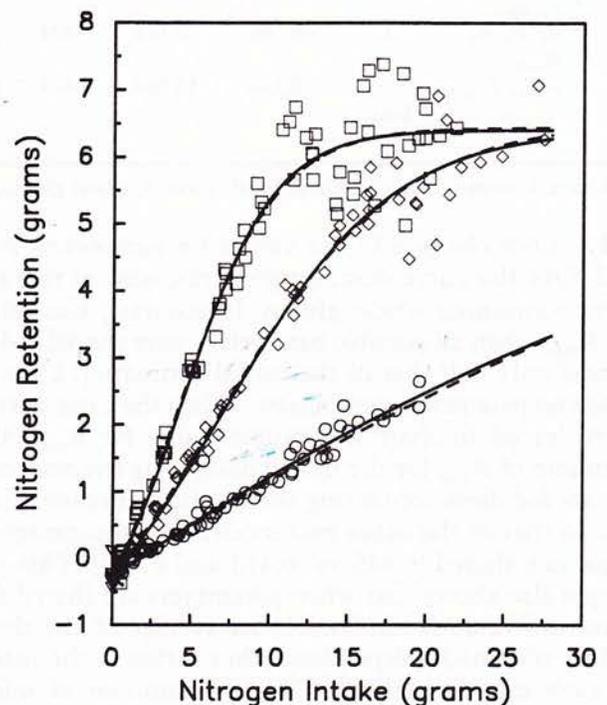


FIGURE 2 Nitrogen retention of rats fed diets containing graded levels of casein, peanut meal or wheat gluten. The lines are the best fits without parameter sharing (—) or with parameters sharing (---). (X) Protein-free; (□) casein; (◇) peanut meal; (○) wheat gluten.

the highest level of wheat gluten had an average nitrogen retention of 2.27 g, while the values for rats fed diets containing similar dietary concentrations of protein from peanut meal or casein were 5.97 and 6.37 g, respectively. When the curves were fit separately, no data were available with which to estimate the value for R_{\max} for the model describing the response of rats fed diets containing wheat gluten. When parameter sharing was used, data from rats fed diets containing casein and peanut meal allowed the program to converge on a reasonable estimate for R_{\max} . The estimate of R_{\max} for rats fed diets containing wheat gluten changed from 12.936 g, without the use of parameter sharing, to 6.445 g with parameter sharing. The accuracy of this estimate can be evaluated because rats fed diets containing high dietary protein concentrations from both casein and peanut meal reached a response plateau. The average nitrogen retention of the animals that appeared to reach an asymptote (as determined by breakpoint analysis) was 6.25 g ($n = 40$), which compares favorably to the pooled estimate of R_{\max} from the logistic model (6.445 ± 0.079 g).

While the value for parameter b could be fixed at the response of the rats fed a protein-free diet, the estimate of parameter b based on a pooled analysis of the data accurately described the response of rats fed a protein-free diet. In this case the average nitrogen retention of rats fed the protein-free diet (-0.27 g) was similar to the logistic model's estimate of parameter b (-0.261 ± 0.045 g).

Use of nonlinear models allows the determination of the relative value of protein sources with respect to their specific applications. Two common applications might involve the determination of the relative values of protein sources for maintenance or growth (19, 20). With casein as the standard, the relative values of peanut meal and wheat gluten can be calculated (Table 4). The relative value of peanut meal for either maintenance or growth was 58% that of casein. This was to be expected since these two curves differed only with respect to the value for parameter d , the scaling parameter. In contrast, the relative value for wheat gluten was highest for maintenance (33%) and slowly decreased as nitrogen retention increased.

Since the relative value of a protein depends on its intended use (i.e., maintenance or gain), this raises questions concerning the current system used to determine protein quality. The current official method, protein efficiency ratio (PER), determines protein quality by feeding rats a diet containing the protein source to be tested at a dietary protein concentration of 10%. For a poor-quality protein this might evaluate the quality of the test protein for its ability to maintain the weight of the test animals. In contrast, at a dietary concentration of 10% a good-quality protein might promote maximum weight gain. That test would be an evaluation of the ability of that protein to promote growth. Since the relative amino acid patterns required

TABLE 4

Nitrogen intakes and relative values required to achieve 0 or 95% of the maximum predicted nitrogen retention based on the four-parameter logistic equation¹

Protein source	Percent of R_{\max}		
	0	50	95
Casein			
Nitrogen intake, g	0.56	5.67	14.18
Relative value	100.0	100.0	100.0
Peanut meal			
Nitrogen intake, g	0.95	9.69	24.26
Relative value	58.51	58.51	58.44
Wheat gluten			
Nitrogen intake, g	1.68	26.86	92.80
Relative intake	33.08	21.11	15.28

¹The model used was that of fit number 5 from Table 2.

for growth and maintenance of the rat appear to differ (21), an evaluation of these two protein sources using the PER method may actually be an evaluation of two different nutrient requirements.

These results indicate that the value of a protein source depends on the growth rate of the animal. Simultaneous analysis of dose-response curves appears to provide an accurate description of the entire response curve while still allowing statistical analysis of the data. The methods described here appear to overcome some of the shortcomings previously associated with use of nonlinear models in protein quality evaluations (5, 16).

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