Chapter 9

USE OF A FOUR PARAMETER LOGISTIC EQUATION AND PARAMETER SHARING TO EVALUATE ANIMAL RESPONSES TO GRADED LEVELS OF NITROGEN OR AMINO ACIDS

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I. DIMINISHING RETURNS AND DOSE–RESPONSE RELATIONSHIPS

A. RECTILINEAR VS CURVILINEAR APPROACHES

The response of animals to graded levels of nitrogen or amino acids has been considered a linear phenomenon in the past. Currently, many of the swine growth models are still based on linear improvements in growth as increments of amino acids are added to a diet. A desirable feature of the statistical approach to defining a linear-plateau relationship is that a single nutrient level is defined as the requirement to maximize growth. A constant efficiency of nutrient use is assumed, and when the requirement is met, the

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efficiency suddenly falls to zero at the plateau (break-point or bent-stick approach). However, several investigators have shown that rats and pigs respond to increments of amino acids in a curvilinear fashion (Finke *et al.*, 1987b; Fuller and Garthwaite, 1993; Gahl *et al.*, 1991, 1994; Mercer *et al.*, 1987; Robbins *et al.*, 1979). Because of the curvilinear response to increasing nutrient intake and the asymptotic plateau (a result of using nonlinear models) defining requirements for maximum gain is difficult. Setting a point on the curve as the requirement becomes arbitrary. Even though the task of describing and interpreting curvilinear responses to nutrient inputs is more complex than the rectilinear approach, the curvilinear approach broadens the scope of information obtained in experiments conducted to compare protein qualities and responses to dietary additions of amino acids.

The shape of the dose–response relationship has been an issue of debate. A theory has been proposed where a single animal's response to graded levels of amino acid was linear and, as a result of pooling a group of these individual linear responses, a curvilinear relationship is observed (Fisher *et al.*, 1973). The original data often used to support this theory was derived from egg production responses of laying hens. Egg output is a discrete variable while growth or protein accretion is a continuous variable; egg production may not be applicable to growth or protein accretion. Fuller and Garthwaite (1993) examined the nitrogen retention response of individual pigs to graded levels of nitrogen. A curvilinear response was observed. Implicit in the curvilinear response is the decreasing efficiency of nutrient use as the maximum gain of the animals is approached. Because of the decreased efficiency of nitrogen utilization near maximum gain, using a break-point or rectilinear estimate of the requirement would underestimate the actual requirement by 25% (Fuller and Garthwaite, 1993). The decreasing improvement in response as equal additional increments of nutrient are added to a diet is referred to as diminishing returns (Parks, 1970, 1982).

In addition, the concept of linear responses to graded levels of nutrient input is not consistent with the kinetics of enzymes or enzyme systems. A curved response for individual animals would be expected just as a Michaelis–Menton relationship is expected with increasing substrate concentration for an enzyme. Curved responses have been observed when lysine α-ketoglutarate reductase activity (a simple enzyme system) and lysine oxidation to CO_2 (a more complicated pathway) were measured (Blemings *et al.*, 1994). Lysine metabolism in liver homogenates may not be directly related to whole animal responses (an animal is not a big enzyme). An animal is a system of pools and fluxes, however, and there does not appear to be a set of discrete on/off switches.

B. FOUR PARAMETER LOGISTIC EQUATION—A NONLINEAR APPROACH

Diminishing returns have implications in evaluating protein quality and in determining amino acid requirements. If the diminishing returns components of response curves differ (different response curve shapes) one would expect the relative values (Hegsted *et al.*, 1968) of proteins to differ over the range of the response. Therefore, rather than comparing proteins at equal intakes, dose–response relationships could be defined for several proteins (or protein mixtures) and relative values could be compared at specific levels of performance. Attempting to characterize enzyme kinetics by measuring the rate at a single substrate concentration would be futile. On the contrary several substrate concentrations are required to define the velocity vs substrate relationship. Therefore, several levels (usually 6 to 12) of nitrogen or amino acids would be required to define diminishing returns responses.

A logistic equation has been used to describe the response of animals to graded levels of nitrogen or amino acids which takes into account the diminishing returns response (Finke *et al.*, 1987a,b, 1989; Gahl *et al.*, 1991, 1994). The logistic equation used previously is defined as follows (Gahl *et al.*, 1991).

$$r = \frac{R_{max} + (b\ (1 + c) - R_{max})\ e^{-kI}}{1 + c \times e^{-kI}},$$

where r is gain at intake "I" (nitrogen or weight), I equals nitrogen or amino acid intake, b is the y intercept (response to zero nutrient), R_{max} is the response maximum (at infinite intake), c is the parameter related to response curve shape, and k is the parameter related to response curve scale.

In order to test differences among curves using this equation, parameter sharing has been used (DeLean *et al.*, 1978; Finke *et al.*, 1987a). The parameters for the response curves are estimated simultaneously and therefore can be forced to share common values based on biological considerations or based on statistical evaluation. For example, the response maximum for a given set of animals would be expected to be the same so R_{max} could be forced to a common value for all curves. The parameters c and k can be shared based on a simple t test between pairs of estimates; the pair with the least significant t test would be forced to a common value in the following fit. An extra sum of squares test is used to test the effect of the constraints (parameter sharing) on the fit (Draper and Smith, 1981). The procedure is similar to performing backward elimination in multiple regression. Parameter sharing allows pooling of data across curves to estimate parameters such as R_{max} (a region of the curve where the greatest variability occurs). Therefore more precise parameter estimates will be made. Parameter sharing is also a more powerful method of testing differences among curves compared to using the confidence limits for the parameter estimates. The NLIN procedure of SAS has been used to estimate the parameters of the logistic equation using parameter sharing to test the differences among curves (SAS, 1982).

II. DIMINISHING RETURNS AND PROTEIN QUALITY

A. RELATIVE VALUES CHANGE WITH RESPECT TO CURVE SHAPE

Finke *et al.* (1987b) have compared the protein qualities of five nitrogen sources using the logistic equation and parameter sharing. Corn gluten meal (CGM), Mormon cricket meal (MCM), and three CGM–MCM mixtures (40% CGM–60% MCM, 50% CGM–50% MCM, and 60% CGM–40% MCM) were fed at 12 levels. The response to these nitrogen sources is shown in Fig. 1. The response to increasing nitrogen intake is curvilinear and is significantly different for each source of nitrogen. The three mixtures when fed to rats resulted in different response curve shapes but not response curve scales. The differences in protein quality that are due to curve shape is illustrated by comparing the relative values of the protein mixtures.

The relative values of the proteins were compared by calculating the intake required for specific levels of performance. Using the 40 CGM–60 MCM mixture as a reference, the relative values of the other proteins were calculated as the intake required for the reference protein divided by the intake required for the test protein (Proteins that required a higher intake at a specific response level had a lower relative value.) The relative values are shown graphically in Fig. 2. When rats were fed diets containing CGM as the protein source, a higher relative value was observed for maintenance (48% for R_{max} = 0) compared to growth (35% for R_{max} = 95%). Similar observations were made when rats were fed MCM; the relative value decreased from 111% at maintenance to 82% near maximum gain. However, when mixtures of the two protein sources were fed the relative values increased from maintenance (70%) to growth (90%). The change in relative values of proteins from maintenance to maximum gain suggests that a single estimate of protein quality at a defined intake would be misleading. Evaluating protein quality at a defined level of performance (by comparing relative intakes) would be a more accurate method.

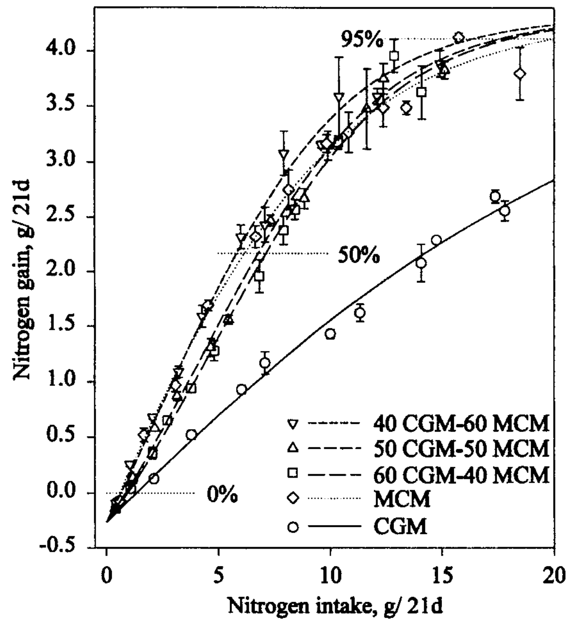

FIG. 1. Body nitrogen gain (g) vs nitrogen intake (g) over 21 days for groups of four rats fed graded levels of corn gluten meal (CGM), Mormon cricket meal (MCM), or CGM–MCM mixtures. Lines are the best fits to the data using the logistic equation (Finke *et al.*, 1987b). Each data point represents the mean ± SEM of four rats.

B. PROTEIN QUALITY IMPROVEMENT BY ADDITION OF THE LIMITING AMINO ACID

Addition of the limiting amino acid is one method of improving the quality of a protein. Methionine (0.4%) was added to the MCM to determine the effect of adding a limiting amino acid to a protein source. The methionine supplementation improved growth and nitrogen gain which is illustrated by the changes in both response curve scale and response curve shape; the c and k parameters were significantly different (Finke *et al.*, 1987b). Using MCM + Met as the reference, MCM alone was always a lower quality protein and the relative value decreased as higher levels of nitrogen were fed (from 90 to 62%). The different response curve shape and scale suggest that MCM + Met when fed to rats results in a different diminishing returns response. The diminishing returns responses can be compared by examining the first derivative of the response curves (Fig. 3). The slope (dr/dI) of the response curve is termed marginal efficiency and reflects the efficiency of use of a "small" increment of nitro-

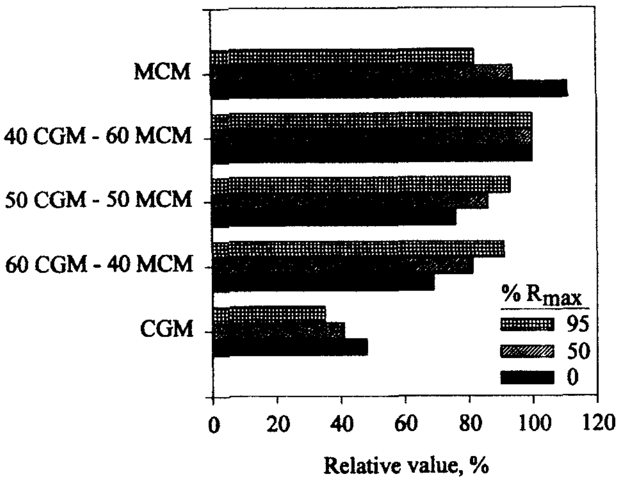

FIG. 2. Relative values of corn gluten meal (CGM), Mormon cricket meal (MCM), and CGM–MCM mixtures at 0, 50, or 95% of R_{max} (Finke *et al.*, 1987b). The 40% CGM–60% MCM protein mixture was used as the control (relative value = 100%). The relative values of the other proteins and protein mixtures were calculated as the nitrogen intake required to achieve a specific nitrogen gain for the control mixture divided by the intake required for the identical response by rats fed the test proteins.

gen added to a diet. When MCM is fed to rats, diminishing returns are observed at intakes near zero (maximum efficiency of nitrogen gain was 47%) and the efficiency of nitrogen use rapidly declines and approaches zero as maximum gain is approached. However, when MCM is supplemented with methionine, the efficiency of nitrogen gain increases to a maximum of 60% at 25% of R_{max} and then declines as R_{max} is approached. The curvilinear response and decreasing efficiency could be due to a change in the limiting amino acid as intake is increased since the pattern of amino acid requirements for maintenance is clearly different than the requirement for maintenance + growth (Benevenga *et al.*, 1994). The change in limiting amino acid could also explain the change in relative values of proteins from maintenance to maximum gain. Amino acids could be used with different efficiencies and could evoke a different diminishing returns response when fed to rats.

III. RESPONSE OF RATS TO EACH INDISPENSABLE AMINO ACID

A series of crystalline amino acid diets were used to examine the response of rats to each indispensable amino acid when limiting (Gahl *et al.*, 1991). The limiting amino acid was fed at 10 levels [0–150% of the National

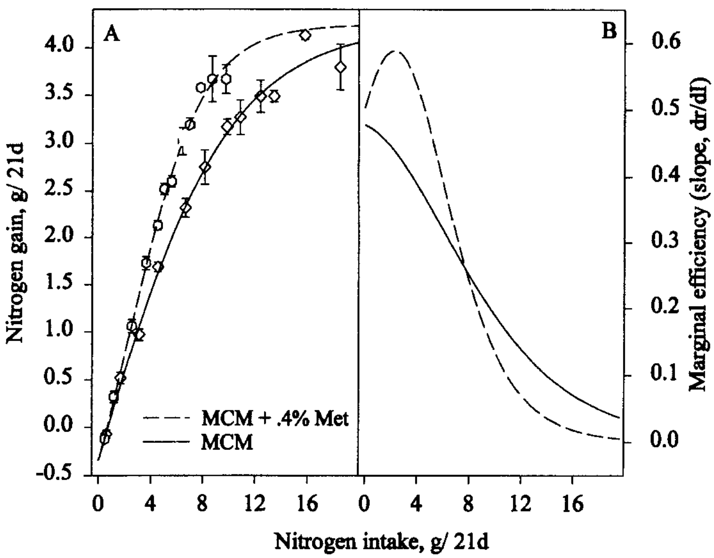

FIG. 3. (A) Body nitrogen gain (g) vs nitrogen intake (g) over 21 days for groups of four rats fed graded levels of Mormon cricket meal or Mormon cricket meal with supplemental methionine. Lines are the best fits using the logistic equation (Finke *et al.*, 1987b). Each data point represents the mean ± SEM of four rats. (B) Marginal efficiency (*dr/dI*) of nitrogen intake used for nitrogen gain. The marginal efficiency reflects the efficiency of utilization of an increment of nitrogen added to the diet.

Research Council (NRC), 1978] while the other amino acids were included in the diet at 35% (35–185%) above the relative level of the limiting amino acid. By using a 35% excess of the other amino acids, the amino acid of interest should remain first limiting. The 100 dietary treatments were fed for 21 days and allowed comparison of the dose–response relationships for each indispensable amino acid. The logistic equation was used to describe the response and parameter sharing was used to test differences among curves. The response curves for four of the amino acids (lysine, methionine + cystine, threonine, and tryptophan) are shown in Fig. 4. Marginal efficiency was used to examine the efficiency of amino acid use and also the magnitude of the diminishing returns component. The efficiency for total sulfur amino acids is similar to that observed for MCM and begins to decline at very low levels of intake. Lysine, threonine, and tryptophan are utilized with a lower efficiency. However the shape of the response curves differ compared to the curve for total sulfur amino acids; there is a maximum at 35% of the requirement and then the efficiency rapidly declines and approaches zero. Diminishing returns impact on responses over at least the upper 60% of the response curve. In summary, the indispensable

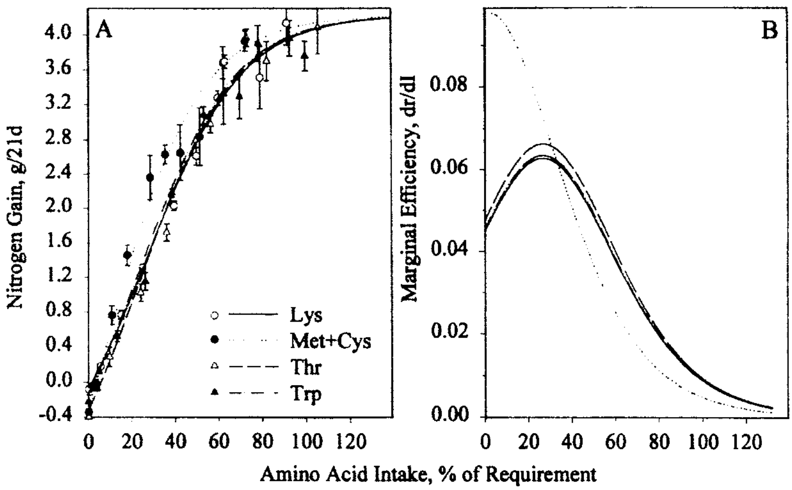

FIG. 4. (A) Nitrogen gain response curves generated using the parameter estimates for the logistic equation for rats fed diets limiting in one indispensable amino acid: lysine, methionine + cystine, threonine, or tryptophan (Gahl *et al.*, 1991). The mean ± SEM for each group of four rats was plotted for each dietary amino acid concentration. (B) Marginal efficiency (*dr/dI*) of the limiting amino acid for nitrogen gain: lysine, methionine + cystine, threonine, or tryptophan. The marginal efficiency reflects the efficiency of utilization of an increment of limiting amino acid added to the diet.

amino acids are used with different efficiencies when limiting and have different diminishing returns responses.

Maximum gain requirements for nitrogen or amino acid intake are more difficult to estimate because of the curvilinear nature of the dose–response relationship compared to a break-point approach which can be used to estimate a single requirement. R_{max} is an asymptotic plateau and theoretically requires infinite intake. An arbitrary decision must be made to estimate the concentration that defines maximum gain. The response curve can be used to estimate the intake required for the chosen level of performance. The required level of intake begins to rapidly increase as levels of performance above 95% R_{max} are attempted. Therefore, 95% R_{max} has been arbitrarily chosen to define the amino acid requirements of the rat using the response curves for each indispensable amino acid (Benevenga *et al.*, 1994). The requirements for 95% R_{max} weight and nitrogen gain are shown in Table I. As expected the requirements for nitrogen gain are 0–44% higher compared to the requirements for weight gain.

An assumption made in using a break-point approach is that the nutrient is used with a constant efficiency. Since the response is curvilinear, the requirement for maximum gain would be underestimated. The amino acid

TABLE I

DIETARY AMINO ACID CONCENTRATION (g/100 g) REQUIRED FOR 95% R_{max} FOR GROWTH OR NITROGEN GAIN IN RATS[a]

	NRC 1978	Weight gain	Nitrogen gain	Ratio nit/weight[b]
Arg	0.60	0.43	0.62	1.44
His	0.60	0.28	0.33	1.18
Ile	0.50	0.62	0.86	1.39
Leu	0.75	1.07	1.30	1.21
Lys	0.70	0.92	1.11	1.21
Met + Cys	0.60	0.97	1.21	1.25
Phe + Tyr	0.80	1.01	1.32	1.31
Thr	0.50	0.62	0.73	1.18
Trp	0.15	0.20	0.20	1.00
Val	0.60	0.74	0.92	1.24

[a] The amino acid requirements were calculated based on the parameters of the logistic equation (Benevenga *et al.*, 1994).

[b] The ratio was calculated as the requirement for nitrogen gain divided by the requirement for weight gain.

would also be expected to be used with the highest efficiency when limiting. Therefore, if a series of experiments were conducted to estimate requirements for amino acids, one might expect that the diet formulated based on the new requirements would not support maximum gain since the amino acids would now be colimiting and would be used with a lower efficiency. Experiments have been conducted to estimate requirements for guinea pigs using a linear approach to estimate each requirement. When a diet was formulated with the estimated requirements, an additional 30–50% of the amino acid mixture was required to obtain maximum gain (Blevins, 1983; NRC, 1995). Although the mixture estimated using the logistic curves (Benevenga *et al.*, 1994) has not been tested, the requirements are estimated taking into account diminishing returns and would not be expected to be underestimated to the same magnitude. However, the requirements estimated from the logistic curves were obtained from rats fed diets that were first limiting in the specific amino acids.

The curvilinear response of animals to graded levels of nitrogen or amino acids necessitates the description of the dose–response relationship. The relative values of proteins differ from maintenance to maximum gain if the shapes of the curves or the magnitude of the diminishing returns components differ. This implies that there is not a single "protein quality" that can be associated with a particular source of nitrogen (protein). Proteins may have a higher quality for maintenance and a lower quality for growth compared to a reference protein. The change in relative values from maintenance to maximum gain may be due to a shift in the limiting amino acid. Different limiting amino acids are used with different efficiencies and have different diminishing returns components.

Because of the curvilinear nature of the dose–response relationship, amino acid requirements should be estimated taking into account the decreasing efficiency associated with diminishing returns. Comparing sources and levels of proteins and amino acids taking into account diminishing returns responses has implications in swine nutrition. Diet formulation decisions should be made based on the economic value of adding increments of amino acids near maximum gain. When the linear–plateau approach is used the optimum level of nitrogen or amino acid to feed is predicted at the "break-point" which corresponds to maximum gain. However, considering diminishing returns the optimum feeding level may not be at maximum gain. The response curve and current prices could be used to predict the optimum level of inputs to feed which would maximize economic return.

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