APPLICATION OF A COMPUTER PROGRAM FOR NONLINEAR REGRESSION ANALYSIS TO RUMEN DIGESTION STUDIES

(APLICACION DE UN PROGRAMA DE REGRESION NO LINEAL A ESTUDIOS DE DIGESTION EN EL RUMEN)

*By S. López **
*and J.P. Barrio ***

Key words: Rumen digestion kinetics, nylon-bag technique, nonlinear regression, model, BASIC.
Palabras clave: Cinética de digestión en el rumen, técnica de las bolsas de nylon, regresión no lineal, modelo, BASIC).

SUMMARY

A micro-computer program in described to estimate the parameters in nonlinear models by a weighted least squares procedure. The program is tested using data from "in situ" digestion experimental trials. Ruminal nitrogen degradation curves of two concentrates and rumen cell wall disappearance curves of two forages have been fitted by various methods, mainly by logarithmic linear transformations *(L-T)* and by a direct nonlinear least squares method *(NLIN)*. Using the coefficient of determination as criterion of the best overall fit, *NLIN* methods seem to provide a better fit than *L-T* methods, although in the case of concentrates *NLIN* methods did not show any advantage over *L-T* methods.

RESUMEN

Se describe un programa en BASIC que permite la estimación de parámetros de modelos no lineales mediante un procedimiento de mínimos cuadrados ponderados.

* Departamento de Producción Animal. Universidad de León. 24071 León.
** Departamento de Fisiología Farmacología y Toxicología. Universidad de León. 24071 León.

An. Fac. Vet. León. 1989, 35, 187-192

Mediante la técnica de incubación ruminal "in situ" se obtuvieron datos de desaparición del nitrógeno de dos concentrados, así como de degradación de la pared celular de un heno y un ensilado. Estos datos fueron ajustados al tiempo de incubación mediante un modelo exponencial, y los parámetros fueron estimados de dos formas: a) tomando logaritmos de la variable dependiente para transformar el modelo en una línea recta *(L-T),* y b) por un método de regresión no lineal por mínimos cuadrados *(NLIN)* empleando nuestro programa. Tomando el coeficiente de determinación de la regresión como criterio para establecer la bondad de ajuste de cada método, se observó que *NLIN* permite un mejor ajuste que *L-T,* aunque en el caso de los concentrados el procedimiento *NLIN* no mostró una ventaja significativa sobre el método *L-T.*

INTRODUCTION

In many rumen studies, data are collected as a function of some condition such as time, and these experimental data often show a nonlinear dependence on the observed values. Most times, the performance of these models requires the use of regression procedures to estimate the parameters of the equations.

Various methods of fit have been used to solve these nonlinear relationships. Direct least squares (LS) regression procedures [2,3] seem to be the most adquate but they require the use of computers in order to estimate the parameters after several cycles of calculation by iterative methods.

In this paper a computer program is described to estimate parameters from nonlinear models by a weighted LS procedure. Although it has been applied to rumen digestion kinetics, it can readily be applied to any nonlinear regression problem.

PROGRAM DESCRIPTION

The program has been written in IBM-PC BASIC. It is able to fit any equation to a set of data showing nonlinear dependence on the observed values. It allows to obtain weighted LS estimates whenever the type of error distribution is specified and the values of weight variable for each case are provided. The general flowchart of the program is shown in Figure 1.

Parameters are estimatd in successive iterations following the Marquardt's algorithm. It is necessary to supply initial values for the parameters. With these starting, non-optimal values, an initial function is described and the initial residual sum of squares (RSS) is calculated. The degree of deviation between these initial values and those of the optimum function is estimated by Taylor series [5]. Although this procedure requires the partial derivatives of the fitted function to be evaluated, analytical expressions for these derivatives need not be supplied as they are approximated by numerical differentiation.

The system assumes that the best estimate has occurred when the relative change in the RSS is less than or equal to the convergence criterion value included in the programa. Standard errors (SE) and the asymptotic correlation matrix of the parameter estimates are obtained by solving the array of the sums of cross-products of the derivatives by the matrix inversion method [5].

The inclusion of a robust weighting procedure results in much improved estimates for the parameters either when observations outside the error range of the other cases (outliers) do appear, or when SE of the parameter estimates are too high.

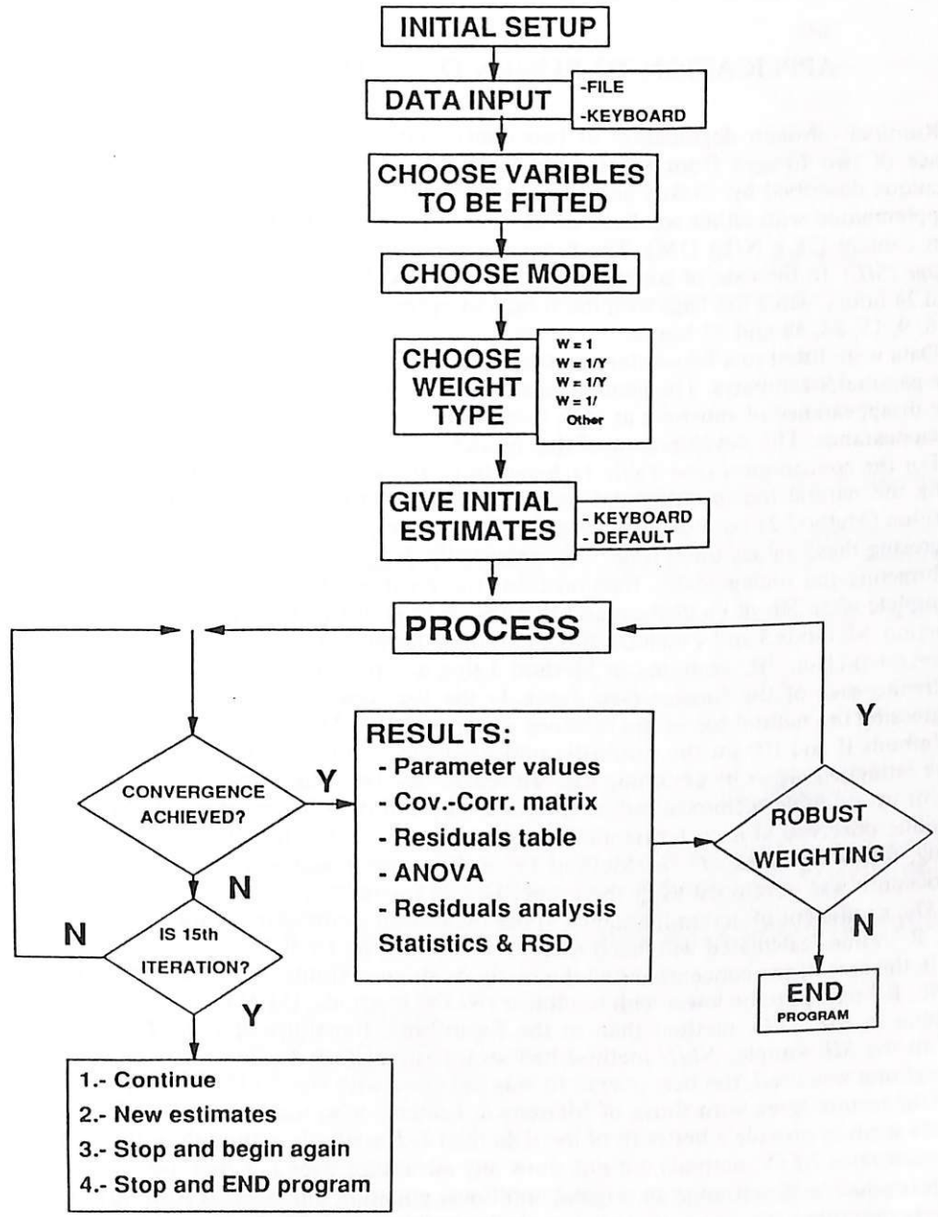

Figure 1.- Flow chart of NLIN program.

Statistics derived from the output are enough to determine how accurate are the parameter estimations themselves (SE) and also to evaluate the goodness-of-fit and the suitability of the model used when compared with others *(F* statistic, R^2*)*. Moreover, the program supplies a fully detailed statistical analysis of the residuals distribution (RSD, Durbin-Watson statistic).

APPLICATION TO RUMEN DIGESTION KINETICS

Ruminal nitrogen degradation of two concentrates and rumen cell wall disappearance of two forages from nylon bags have been studied following the nylon-bag tecnique described by Ørskov and McDonald [4]. The concentrates were crushed barley supplemented with either soy-bean meal (SSC) or urea (USC) to have the same nitrogen content (28 g N/kg DM). The forages were a meadow hay *(HAY) and a herbage silage (SIL).* In the case of concentrates, "in situ" incubation times were 3, 6, 9, 12, 18 and 24 hours, while the bags with the forage samples were removed from the rumen at 3, 6, 9, 15, 24, 48 and 72 hours.

Data were fitted to a first-order kinetic model using various techniques for obtaining the parameter estimates. The general equation of the model is $dD/dt = -kD$, where D is the disappearance of substrate at each incubation time(t) and k is the fractional rate of disappearance. The development of this model results in a exponential equation.

For the concentrates (see Table 1), logarithmic transformation was used by determining the natural log of either the residue (Method 1) or the potentially degradable residue (Method 2) both of them remaining at the end of each incubation period, and regresing these values upon time. Teh potentially degradable residue was obtained by subtracting the undegradable fraction from the residue, assuming that digestion was complete after 24h of incubation and that the residue at this time is the undegradable fraction. Methods 3 and 4 include direct nonlinear iterative *LS* regressions of the model $D = a + b\ (1 - e^{-kt})$[4], assuming in Method 3 that $a + b = 100$.

In the case of the forages (see Table 1) the log- linear transformation was used regressing the natural log of the residues either without (Method I) or with correction (Methods II and III) for the ruminally undegradable residue. This indigestible residue was estimated either by assuming the 72h residue as the indigestible fraction (Method II) or by a double reciprocal model (Method III), and then it was subtracted from the residue observed at each fermentation time to estimate the digestible residue remaining, following Mertens [1]. In Method IV, a direct nonlinear iterative LS estimating procedure was developed to fit the model $D = b(1 - e^{-k(t-L)})$.

The coefficient of determination (R^2) has been used as measure of the goodness-of-fit. R^2 values calculated with each method are shown in Table 1.

In the case of the concentrates all the methods showed similar R^2 values although in *USC,* R^2 tended to be lower with nonlinear *(NLIN)* methods. IM *HAY,* R^2 values were higher in the *NLIN* method than in the logarithmic transformation *(L-T)* methods. With the *SIL* sample, *NLIN* method had an intermediate R^2 value but when robust weighting was used, the best overall fit was obtained with the *NLIN* method.

Our results agree with those of Mertens & Loften [2] who indicated that *NLIN* methods seem to provide a better fit of the data than *L-T* methods, although in the case of concentrates *NLIN* methods did not show any advantage over *L-T* methods.

It is possible to rearrange an original non-linear equation into a linear form (as in *L-T* methods), these transformations being algebraically correct, but they are inappropiate for analysing experimental data since they result in non-standard error distributions,

TABLE 1
Coefficients of determination from logarithmic transformations and nonlinear methods

	CONCENTRATE	CONCENTRATE
	USC	**SSC**
Method 1.[a]	0.9316	0.9829
Method 2[b]	0.9401	0.9272
Method 3[c]	0.8854	0.9793
Method 4[d]	0.8940	0.9793
	HAY	**SILAGE**
Method I[e]	0.9196	0.9298
Method II[f]	0.9768	0.9857
Method III[g]	0.9579	0.9697
Method IV[h]	0.9975	0.9416

[a] $\ln R = \ln B - kt$, where R = N residue remaining at each incubation time (t). If $B < 100$ then $A = 100\text{-}B$, else $B = 100$, $A = 0$ and L is t when $R = 100$.

[b] $\ln(R\text{-}U) = \ln B - kt$, where R = N residue remaining at each incubation time (t) and U = N residue remaining after 24h. If $B < (100\text{-}U)$ then $A = (100\text{-}U)\text{-}B$, else $B = 100\text{-}U$, $A = 0$ and l is t when $R = 100$.

[c] $D = 100 - Be^{-kt}$ where D = N disappearance from the bags at each incubation time (t) and $A + b = 100$.

[d] $D = A + B(1\text{-}e^{-kt})$, where D = N disappearance from the bags at each incubation time(t).

[e] $\ln R = \ln B - kt$, where R = CW residue reamining at each incubation time(t) if $B < (100\text{-}U)$ then $A = (100\text{-}U)\text{-}B$, else $B = 100\text{-}U$, $A = 0$ and L is t when $R = 100$. U = CW residue remaining after 72h.

[f] $\ln(R\text{-}U) = \ln B - kt$. where R = CW residue reamaining at each incubation time(t) and U = CW residue remaining after 72 h. if $B < 100\text{-}U)$ then $A = (100\text{-}U)\text{-}B$, else $B = 100\text{-}U$, $A = 0$ and L is t when $R = 100$.

[g] $\ln(R\text{-}U) = \ln B - kt$, where R = CW residue remaining at each incubation time (t) and U = undegradable CW residue estimated by a double reciprocal approach. If $B < (100\text{-}U)$ then $A = 100\text{-}U\text{-}B$, else $B = 100\text{-}U$, $A = 0$ and L is t when $R = 100$.

[h] $D = B(1\text{-}e^{-k(t-L)})$, where D = CW disappearance from the bags at each incubation time (t). If $L < 0$ then $D = A + B\ (1\text{-}e^{-kt})$.

Parameters:
A = Fraction soluble or readily degraded.
B = Fraction slowly degraded at a rate k.
k = Fractional degradation rate for B fraction.
L = Lag time.

and these are needed for *LS* procedures. The type of error distribution can change when the axes have been distorted by transformation to the linear form. Moreover, by using these graphical techniques it is difficult to obtain any meaningful indication of

the accuracy of the calculated parameters, and these estimates are more variable than those obtained by direct *LS*.The *NLIN* procedure also simultaneously derives all parameter estimates while other methods require a sequential determination of specific estimates. So, in L-T methods the undegrable fraction, the digestion rate, and the lag time are calculated in successive steps. Moreover, the inclusion of certain assumptions in the development of the *L-T* methods would result in biased estimated. The final results depend on the accuracy of the previous estimations and these often are very sujective (e.g. the choice of the end point for the estimation of the undegrable fraction) [1].

On the other hand, the *NLIN* procedure is more flexible and it can be used to fit any model, although starting values close to the final solution are needed for the method to reduce the computations required and to increase the likelihood of finding a global and accurate solution. Additionally, this method is sensitive to variation in the data set what can lead to unrealistic estimates, unless certain constraints are specified for the parameters. This occurs with *USC* in the *NLIN* methods, where if it is not assumed that a + b = 100, potential degradation values higher than 100 can be obtained.

An appropiate number of data points in relation to the number of parameters to be estimated would avoid this problem. In this sense, *NLIN* methods requires more cases than *L-T* methods, but less than other methods like curve peeling.

We can conclude that *NLIN* method would be the most accurate and the least biased procedure for describing rumen digestion kinetics.

The authors would be glad to supply a listing of the program to interested readers.

REFERENCES

1) MERTENS, D.R. (1973). Application of Theoretical Mathematical Models to Cell Wall Digestion and Forage Intake in Ruminants. Ph.D. Dissertation Thesis, Cornell University. New York.
2) MERTENS, D.R. & LOFTEN, J.R. (1980). The effect of starch on forage fiber digestion techniques. *J. Dairy Sci., 63:* 1.437-1.446.
3) NOCEK, J.E. & ENGLISH, J.E. (1986). In situ digestion techniques: Evaluation of rate determination procedure. *J. Dairy Sci., 69:* 77-87.
4) ØRSKOV, R.E. & McDONALD, I. (1979). The estimate of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci., Camb., 42:* 499-503.
5) SNEDECOR, G.W. & COCHRAN, W.G. (1981). *Statistical Methods.* 7th ed. The Iowa State University Press, Ames, Iowa.

MORFOLOGICAL STUDY OF A CASE THE HERMAPHRODITISM IN THE *PROCAMBARUS CLARKII* (CRUSTACEA, DECAPODA) FROM ALBUFERE LAKE OF VALENCIA

(ESTUDIO MORFOLOGICO DE UN CASO DE HERMAFRODITISMO EN *PROCAMBARUS CLARKII* (CRUSTACEO, DECAPODO) DE LA ALBUFERA DE VALENCIA)

*By M.M. Almar, C. Soler * and M.D. Ferrando**

Palabras clave: *Procambarus clarkii,* hermafroditismo, gónadas.
Key words: *Procambarus clarkii,* hermaphroditism, gonades.

ABSTRAC

Approximately 7.000 specimens of the crayfish *Procambarus clarkii* from the Albufera lake (Valencia, Spain) were collected and examined, and only one apparent hermaphrodite animal was localized. This animal was studied and its gonads were fixed in Bouin's solution for 2 hours. The posterior genital lobe presented in its proximal zone an altered ovarian tissue and in its distal zone the tecticular acini found were inactive. This observation constitute a real description of a case of autentic hermaphroditism in this specie.

RESUMEN

Hemos recogido y revisado aproximadamente 7.000 ejemplares de la especie *Procambarus clarkii* y de todos ellos sólo uno era aparantemente hermafrodita. Este animal se

Dpto. Fisiología, Farmacología y Toxicología Universidad de León. 24007 León.
* Dpto. Biología Animal Universidad de Valencia. SPAIN

An. Fac. Vet. León. 1989, 35, 193-199