ELSEVIER

Animal Feed Science and Technology 71 (1998) 251–267

ANIMAL FEED SCIENCE AND TECHNOLOGY

# Models of suppressive effect of tannins. Analysis of the suppressive effect of tannins on ruminal degradation by compartmental models

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Accepted 3 September 1997

**Abstract**

Three compartmental models were developed to evaluate the suppressive effects of tannins on the extent and rate of degradation of tree foliage. The first model was based on the assumption that tannins modify the parameters of the degradation kinetics, the second was based on the assumption that tannins bind material, independent of the basic degradation kinetics, and the third combined these two assumptions, i.e., that tannins both suppress degradation and bind free material. Degradation was measured by suspending the samples in dacron bags in the rumens of goats, with or without the inclusion of polyethylene glycol (PEG). It was assumed that PEG annuls the suppressive effect of tannins on degradation of plant components, and hence that the difference between the curves that describe degradation with or without PEG indicates the suppressive effect of tannins present in the foliage. The data on the observed degradation of dry matter, neutral detergent fibre and protein, in four typical Mediterranean forest tree species were fitted by these models. It was found that the combined model fits the data better than either of the other two models. Increasing content of tannins in the foliage was associated with an increase of the bound free material and a decrease in the degradation rate of the degradable matter, but not with an increase of the non-degradable fraction. © 1998 Elsevier Science B.V.

*Keywords:* Tannins; In situ degradation; Compartmental analysis; Ruminants

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*PII* S0377-8401(97)00147-8

## 1. Introduction

Trees and shrubs are important feeds for ruminants in dry environments, but their use is often limited by phenolic compounds such as tannins, which can adversely affect digestibility and intake (Kumar and Vaithiyanathan, 1990; Silanikove et al., 1994). The major negative effect of these compounds results from either formation of the indigestible compounds with food and endogenous protein, or direct inhibition of digestive enzymes (Kumar and Vaithiyanathan, 1990). Inclusion of polyethylene glycol (PEG) with a molecular weight of 4000 in tannin-containing leaf diets for sheep (Pritchard et al., 1992; Terril et al., 1992; Silanikove et al., 1994) improved digestibility, dry matter (DM) intake, and performance. In vitro studies showed that the addition of PEG to plant samples containing tannins, increased the digestibility of DM and protein, (Garrido et al., 1991). This effect of PEG on tannin-containing diets arises because tannins form complexes with PEG more readily than with proteins, and these complexes are stable under the chemical conditions prevailing in the digestive tract (Jones and Mangan, 1977). Recently, an assay of tannin content and their negative effect on ruminal degradation was developed, using the tannins binding capacity of PEG (Silanikove et al., 1996).

To evaluate the suppressive effect of tannins on degradability, we assumed that when 2 g PEG is added to each 5-g sample of dried and ground foliage, the suppressive effect of tannins is completely annulled and hence that the net effect of tannins could be indicated by the difference between samples that were incubated with or without PEG. The effect on the effective degradability ($E$, Ørskov and McDonald, 1979) could be evaluated by the difference between the two values of $E$, obtained in the presence and absence of PEG.

The goals of the present study were: (i) to test the ability of models of the degradation kinetics to fit data of tannin-containing material that disappeared during its incubation for up to 72 h in dacron bags, with or without the presence of PEG, in the rumens of goats, and (ii) to relate the kinetic parameters estimated by these models to the tannin content of the foliage, and to the extent of suppression of degradability.

## 2. Materials and methods

### 2.1. In situ procedures

Degradability of foliage of four species of trees typical of the Mediterranean forest was tested by suspending samples in dacron bags in the rumens of goats (see Setala, 1983 for detailed procedure). Samples of 5 g of dried and ground foliage were incubated either with 2 g PEG 4000 or without PEG, for periods of 0, 3, 6, 9, 12, 24, 36, 48 and 72 h. Preliminary studies showed that increasing the amount of PEG above 2 g per bag did not result in further increase of degradation of any of the tested foliage. Dry matter (DM), neutral detergent fibre (NDF) and crude protein (CP) were determined in the original samples and in residues in the dacron bags. The fraction degraded up to each time point was calculated for each of the three components (in three replicates). The four tree species were: *Calicotome villosa* (CV, Chinese cinnamon), a low tannin species;

Table 1
Composition of foliage of four species of trees typical to the Mediterranean forest (g/kg in dry matter)

| Species[a] | Ash | Protein | NDF | Cellulose | Condensed tannins | Total phenols |
|---|---|---|---|---|---|---|
| CV | 50 | 124 | 555 | 219 | 0.9 | 8.6 |
| CS | 50 | 90 | 575 | 223 | 40 | 17.6 |
| QC | 55 | 66 | 488 | 265 | 99 | 40.6 |
| PP | 55 | 112 | 522 | 152 | 308 | 112.8 |

[a]Species: CV—*Calicotome villosa*; CS—*Ceratonia siliqua*; QS—*Quercus calliprinos*; PP—*Pistacia palaestina*.

*Ceratonia siliqua* (CS, carob tree), a medium tannin species; *Quercus calliprinos* (QC, oak), a high tannin species; and *Pistacia palaestina* (PP, terebinth), a very high tannin species. The contents of ash, cellulose and crude protein (Kjeldahl X 6.25) in the DM component of foliage from these species were determined by standard procedures (AOAC, 1984), NDF was determined according to Van Soest et al. (1991). Condensed tannins (Porter et al., 1986) and total phenolic compounds (Swain and Hillis, 1959) were analysed and expressed as described by Silanikove et al. (1994). The composition of the foliage is presented in Table 1.

### 2.2. Degradability calculations

The kinetics of ruminal degradation of feedstuffs was suggested by Waldo and Smith (1972) to be simple first order, so that the fraction $Y_t$, degraded up to time $t$, is given by:

$$Y_t = A + B * \left(1 - \exp\left(-K_b * (t - L)\right)\right) \quad (1)$$

where: $A$ = the fraction of immediately degraded material, $B$ = the fraction of slowly degraded material, $K_b$ = the fractional rate of degradation of $B$, $L$ = lag time, before $B$ starts to be degraded.

Degradability of feedstuffs, suspended in dacron bags for a series of time in the rumen, has been estimated by this model.

The in vivo effective degradation ($E$) of feed in the rumen was suggested by Ørskov and McDonald (1979) to be a product of the degradation rate, as expressed in Eq. (1), and the rate of passage of material from the rumen ($K_r$). The integration of this function with time gives:

$$E = A + B * K_b/(K_b + K_r) * \exp(-K_r * t) \quad (2)$$

or:

$$E = A + B * K_b/(K_b + K_r) \quad (3)$$

if lag time is not considered.

### 2.3. Compartmental analysis

Eq. (1) was used in Models 1 and 2 for the degradation curve of material with the presence of PEG (HD high degradation curve). When data are obtained for two different degradabilities of the same component in the same feed, e.g., with or without the inclusion of PEG, two sets of parameters need to be estimated. Model 1 was based on

the assumption that tannins interfere with the four kinetic parameters and alter them. Therefore, a second set of parameters $A_1$, $B_1$, $K_{b1}$ and $L_1$ was assigned to compartments 11, 12, and 13, to describe the degradability in the absence of PEG (LD, low degradation curve), in the same way as HD, to give eight parameters arranged in two independent routes for this model as a whole (Fig. 1).

Model 2 was based on the assumption that the tannins are able to bind free material, which was either liberated from the tested sample or available from the surroundings, and they do not affect the kinetic parameters of the HD curve. This assumption calls for an estimation of one amount of material which is degraded with time, and equal in the presence or absence of PEG, and another amount of material which is bound with time

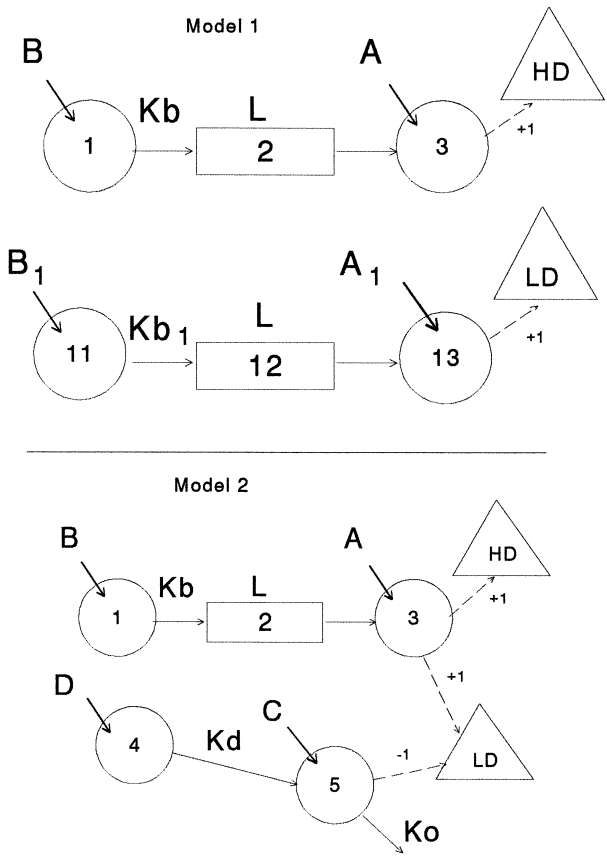


Fig. 1. Compartmental analysis flow charts of models 1 and 2. Circles in the charts are assigned to compartments, with definition of the compartment number. Rectangles are assigned to lag times, defined as delay compartments, and triangles to summing compartments of the accumulated degraded material with the presence (HD) and absence (LD) of PEG. The fractions assigned to the compartments as initial conditions were A, $A_1$ and B, $B_1$ for HD and LD respectively; C and D. The fractional rates Kb, $Kb_1$, Kd, and Ko are defined by arrows, arrows without definition are fixed unity rate fluxes, which are practically instantaneous, and broken-line arrows define information transfer. See text for the definition of initial conditions and fractional rate parameters.

by tannins. The HD curve describes the change with time of the degraded material, and the LD curve describes the change with time of the sum of the degraded material and the negative value of the bound material. The first four parameters were therefore the same as in Model 1. Another set of four parameters was assigned to calculate the variation of the amount of bound material with time: $C$—the readily bound fraction of the sample DM; $D$—the fraction of the sample DM which is bound at a slow rate after the start of the incubation; $K_d$—the rate at which fraction $D$ is bound; and $K_o$—a rate at which the bound material forming fractions $C$ and $D$ is re-liberated. Model 2 includes eight parameters, as in Model 1, but the two routes are connected by the same four parameters that are involved in the descriptions of both HD and LD (Fig. 1).

In Model 2, unlike Model 1, the second set of parameters is related to the first set, and therefore they should be calculated simultaneously. For this purpose, integration of the curves in a compartmental model is more appropriate than in a model based on analytical equations, because it allows any type of mathematical connection between the parameters of the two sets, and the possibility to run the two sets simultaneously. Therefore, all models were designed and analysed as multi-compartmental models, using the CONSAM program (Berman et al., 1962; Boston et al., 1982).

In compartmental analysis terms, Eq. (1) is represented as a model comprising three compartments: compartment 1, to which fraction $B$ is added at $t = 0$, compartment 2, which is a delay compartment between compartments 1 and 3, and compartment 3, to which fraction $A$ is added at $t = 0$. The connections between the compartments are described by a flux from 1 to 2, representing $K_b$, and a one-unit flux from 2 to 3. This one-unit flux transfers degraded material from compartment 2 to compartment 3 at a further delay of one unit (1 h) of time, which should be added to the estimated lag time $L$ Thus, the actual calculated lag time equals $L + 1$. The amount of material accumulated in compartment 3 up to any given time represents the degraded fraction up to that time. The four basic parameters in this model remain $A$, $B$, $K_b$ and $L$, as in the analytical equation, but the solution of the curve of accumulation of degraded material is performed numerically by integrating the change of compartment masses with time.

Flow charts of Models 1 and 2 are given in Fig. 1. The summing compartments of HD and LD are separate in Model 1, each one of them sums the accumulation with time at the end of each route. In Model 2, however, the LD compartment sums the positive value of the degradation route and the negative value of the binding route.

Model 3 (Fig. 2) was based on the assumption that both processes take place, i.e., that tannins both affect the kinetic parameters of the HD curve, and bind free material. The simulation of this situation needed at least 12 parameters: the eight parameters of Model 1 which describe the degradation in the presence and absence of PEG and, in addition, the four parameters that describe the independent binding effect, as in Model 2. The summing compartment of LD sums the positive value of the degradation route and the negative value of the binding route. Unlike model 2, the degradation in the absence of PEG differs from that in the presence of PEG. It was assumed that the difference between the amount of potentially degraded material in the presence and in the absence of PEG represents an amount which is bound to tannins in addition to the amounts of fractions $C$ and $D$. This amount (Pd) was assigned to compartment 15 in the model. It was further assumed that this material could be liberated at a slow rate, similar to the

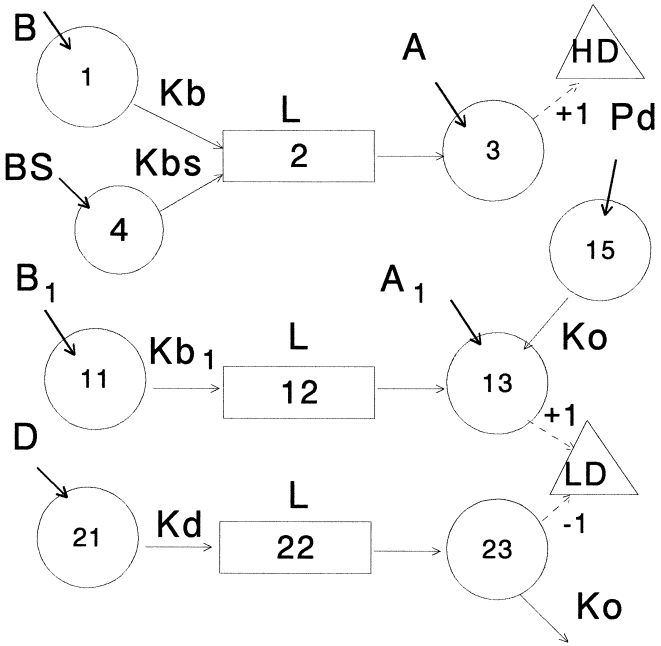


Fig. 2. Compartmental analysis flow chart of Model 3. Circles in the chart are assigned to compartments, with definition of the compartment number. Rectangles are assigned to lag times, defined as delay compartments, and triangles to summing compartments of the accumulated degraded material with the presence (HD) and absence (LD) of PEG. The fractions assigned to the compartments as initial conditions were A, $A_1$ and B, $B_1$ for HD and LD respectively; BS, D, and Pd. The fractional rates Kb, Kbs, $Kb_1$, Kd, and Ko are defined by arrows, arrows without definition are fixed unity rate fluxes, and broken-line arrows define information transfer. See text for the definition of initial conditions and fractional rate parameters.

rate of re-liberation of bound material forming fraction *D*. Therefore, the same rate, $K_o$ was assigned to the outward flux from compartment 23, and to the flux from compartment 15 to compartment 13.

When data were processed by Models 1 and 3, it was evident that the fit of the HD curve for some of the data sets could be improved if the degradation rate of fraction *B* changed with time, as suggested by France et al. (1993). An alternative way to obtain a similar improvement is to divide fraction *B* into two separate compartments, one of which is degraded at a slower rate than the other, where both rates are constant with time. Therefore, two parameters were added in Model 3 to account for the fraction of more slowly degraded material (BS), and for the rate ($K_{bs}$) at which this fraction was degraded. Model 3 included, therefore, a total of 14 parameters.

There is a possibility of more than one model to fit the same data. However, the selection of a model should be assessed by three criteria (Aharoni et al., 1991): (a) goodness of fit, both by visual observation of the curves and a comparison of the sum of squares to that of other alternative models; (b) significance, i.e. the fractional standard deviations (FSD) of the estimated parameters are low; (c) consistency, i.e. the same model should fit all the data sets in a study, and should be consistent with existing

knowledge as for the nature of the tested system. On the assumptions on which Model 3 was based, an alternative model, comprised 14 parameters like Model 3, was constructed. In this model, the fractions $A$, $B$ and BS of the LD curve, but not the rate fluxes $K_b$ and $K_{bs}$, were forced to equal those of the LD curve, and additional fraction was inserted to compartment 13, to account for the difference in potential degradability between the HD and LD routes. This model, however, yielded similar results to those of Model 3, and is not presented here.

### 2.4. Calculations

Because $E$ calculations were based on the calculated values of the LD and HD curves, when these curves fail to fit the data, $E$ calculations might also be inaccurate, especially when there were large differences between calculated and observed values in the first period. Therefore, $E$ was calculated only for Model 3 estimations, which achieved the best fits.

Effective degradability was calculated for Model 3 as suggested by Ørskov and McDonald (1979), but because the kinetic parameters in this model did not allow calculation by Eq. (2), the calculated HD and LD curves were extrapolated to 400 h, and additional time points were added to them in the first 72 h of incubation, to allow numerical integration of $E$, assuming a fractional clearing rate ($K_r$) from the rumen of 0.025 per h.

The fitting of the two curves simultaneously, based on the model parameters, to the data was performed by the CONSAM programme in an iterative process, after initial estimates were assigned to the parameters. The fit was considered completed when no further decrease of the sum of squares of the differences between calculated and observed values could be achieved (for convergence measures which are calculated by CONSAM, see Berman et al., 1962). When the fit had been completed, the sums of squares (SS) were available for the HD curve (SSHD), the LD curve (SSLD), and the entire data set (SST). For comparison between models of the goodness of fit, the ratios of SS of model 1 to those of Model 3, and of those of Model 2 to those of Model 3 were calculated for the three SS parameters, in each of the 12 data sets of components within species. The sum of squares is adjusted in CONSAM to the statistical weights of the data points and is not affected by the number of adjustable parameters in the model (Berman et al., 1962). Therefore, SS values by different models to the same data set are comparable, provided that the statistical weights of the data points are equal among models. Comparisons of these ratios between models, when the SS values for Model 3 were normalised to unity, were performed by means of paired $t$-test analysis.

Because Model 3 includes 14 parameters, and the number of observations in each of the 12 data sets could hardly support this many parameters, a trade-off among related parameters was possible. This possibility was indicated by high correlations between certain parameters, and large standard errors of these parameters.

However, groups of parameters could be referred to the tannin content or to the suppression effect. By summing parameters in such group parameters, a reference to the degradability traits of tannin-containing foliage could be investigated. Four such groups of Model 3 parameters were formed, that refer to the following traits: (i) the difference

in size between the HD and LD readily degradable fractions (RHL); (ii) the difference in size between the HD and LD slowly degradable fractions (SHL); (iii) the ratio of mean degradability rate of the slowly degradable fraction of HD, to that of LD (DHL); and (iv) the size of the independently bound fraction in LD (IBL). These group parameters were defined by the equations:

$$\mathrm{RHL} = A - A_1$$

$$\mathrm{SHL} = B + \mathrm{BS} - B_1$$

$$\mathrm{DHL} = \left(\left(K_{\mathrm{b}} * B + K_{\mathrm{bs}} * \mathrm{BS}\right)/\left(B + \mathrm{BS}\right)\right)/K_{\mathrm{bl}}$$

$$\mathrm{IBL} = D + \mathrm{Pd}$$

The relations between these group parameters and $E$ values obtained from the HD curve and the LD curve, and the difference between $E$ values obtained from the two curves (DIF) were studied, using single and multiple linear regression analysis. All the statistical analyses were carried out using GENSTAT 5 Release 3.2 (Lawes Agricultural Trust, Rothamsted Experimental Station, HARPENDEN, Hertfordshire, UK AL5 2JQ).

## 3. Results

The curve fits to the degradation with time of the DM, NDF, and CP components of the low tannin species CV were good, and similar in all three models. In the cases of other species, however, Models 1 and 2 failed to fit the HD and LD curves to the data. This was more evident for the NDF and CP components than for the DM component. In Fig. 3 are presented curve fits by Models 1, 2 and 3, to the degradation with time of the NDF component of PP, a very high tannin species. For comparison, the curve fit by Model 3 to the degradation of the DM component of CV is also shown (Fig. 3a). It was evidence that the LD curve of Model 1 (Fig. 3b) could not follow the initial accumulation of material in the dacron bag, while the HD curve of Model 2 (Fig. 3c) failed to follow the data. Model 3, however, fitted both HD and LD curves to the data in a much better way (Fig. 3d).

Comparison between models of the SS ratios for the curve fit is presented in Table 2. The ratios of the SS values of Model 1 to those of Model 3, and of those of Model 2 to those of Model 3, are presented with their standard errors. The SST of Model 1 was significantly higher than those of Models 2 and 3, and that of Model 2 tended ($P = 0.06$) to be higher than that of Model 3. However, the comparison between Model 1 and Model 3 indicates large and significant differences in the fit of the LD curve, whereas the differences in the fit of the HD curve were relatively small. The comparison between Model 2 and Model 3 indicate similar ratios, and similar significance of the difference between the two curves.

Fig. 3. Curve fit to the accumulated degradation with time of samples incubated in the presence (rectangles, fitted by HD, solid line) and absence of PEG (triangles, fitted by LD, dotted line). (a) DM component of *Calicotome villosa*, fitted by Model 3; NDF component of *Pistacia palaestina* fitted by Model 1 (b), Model 2 (c), and Model 3 (d).

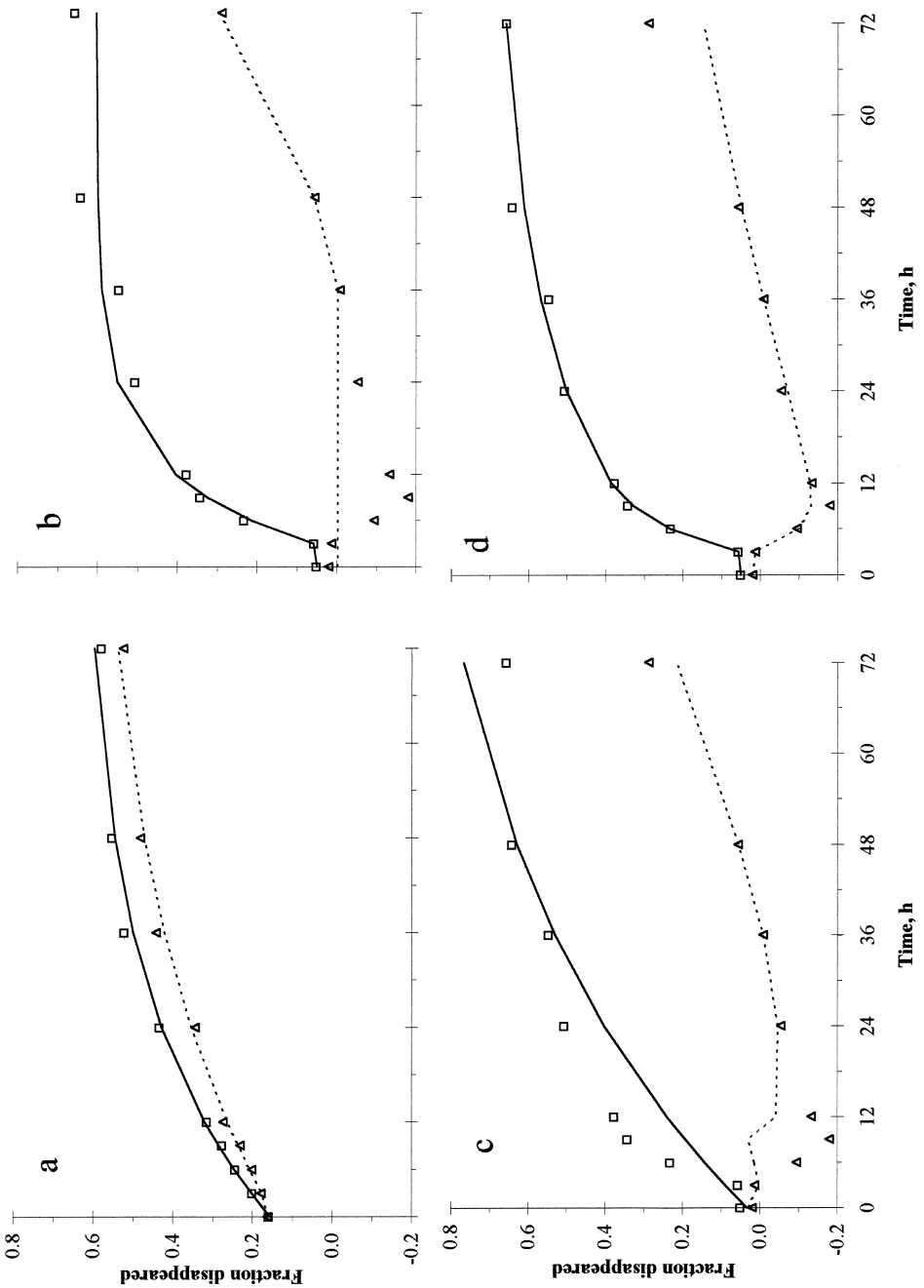
a
b
c
d
Fraction disappeared
Time, h
0.8
0.6
0.4
0.2
0.0
-0.2
0
12
24
36
48
60
72

Table 2
Paired *t*-test ($n = 12$) comparisons of the ratio of sum of squares (SS) in Models 1 and 2, and SS in Model 3. Sum of squares and its SEM are presented for the high degradability curve (SSHD), the low degradability curve (SSLD), and the sum of the two curves (SST). Probability for the differences between models is also presented

| | Ratios to Model 3 | | Probability of the difference | | |
|---|---|---|---|---|---|
| | Model 1 | Model 2 | 1 to 2 | 1 to 3 | 2 to 3 |
| SSHD | $2.91 \pm 1.42$ | $4.62 \pm 1.70$ | 0.102 | 0.202 | 0.057 |
| SSLD | $7.67 \pm 2.29$ | $4.28 \pm 1.56$ | 0.005 | 0.014 | 0.056 |
| SST | $6.37 \pm 2.12$ | $4.23 \pm 1.54$ | 0.019 | 0.028 | 0.060 |

The *E* values for HD and LD, and the values of DIF, the difference between *E* of HD and that of LD, and the values of the group parameters RHL, SHL, DHL, and IBL, are presented in Table 3. The mean value for each of the components across species, and of species across components, are also presented. HD and LD in the NDF were lower

Table 3
Effective degradability estimated for the high degradation curve (HD), low degradation curve (LD), and the difference (DIF) between them, and the values of group parameters calculated from the Model 3 parameters, for DM, NDF, and CP components, in the foliage of trees typical to the Mediterranean forest

| Species[a] | Component | Degradability | | | Group parameters[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | | HD | LD | DIF | RHL | SHL | DHL | IBL |
| CV | DM | 0.439 | 0.383 | 0.056 | −0.005 | 0.077 | 1.367 | 0.082 |
| | NDF | 0.451 | 0.387 | 0.065 | 0.044 | 0.318 | 0.818 | 0.415 |
| | CP | 0.617 | 0.505 | 0.112 | 0.055 | 0.096 | 2.569 | 0.152 |
| CS | DM | 0.525 | 0.380 | 0.145 | −0.005 | 0.574 | 0.094 | 0.570 |
| | NDF | 0.467 | 0.286 | 0.181 | 0.043 | 0.180 | 0.299 | 0.320 |
| | CP | 0.419 | 0.155 | 0.264 | 0.131 | −0.006 | 0.147 | 0.611 |
| QC | DM | 0.487 | 0.261 | 0.226 | 0.053 | 0.299 | 0.037 | 0.565 |
| | NDF | 0.220 | −0.080 | 0.301 | 0.142 | −0.086 | 1.718 | 0.245 |
| | CP | 0.388 | 0.153 | 0.235 | 0.152 | −0.035 | 4.202 | 0.174 |
| PP | DM | 0.606 | 0.324 | 0.281 | 0.001 | 0.177 | 2.053 | 0.446 |
| | NDF | 0.478 | 0.025 | 0.453 | 0.030 | 0.581 | 6.706 | 0.830 |
| | CP | 0.687 | 0.385 | 0.302 | 0.183 | −0.107 | 4.507 | 0.183 |
| Mean | DM | 0.514 | 0.337 | 0.177 | 0.011 | 0.282 | 0.888 | 0.416 |
| Comp. | NDF | 0.404 | 0.154 | 0.250 | 0.065 | 0.248 | 2.385 | 0.452 |
| | CP | 0.528 | 0.299 | 0.228 | 0.130 | −0.013 | 2.856 | 0.280 |
| Mean | CV | 0.520 | 0.425 | 0.078 | 0.031 | 0.164 | 1.584 | 0.216 |
| species | CS | 0.470 | 0.274 | 0.196 | 0.056 | 0.249 | 0.180 | 0.500 |
| | QC | 0.365 | 0.111 | 0.254 | 0.116 | 0.059 | 1.986 | 0.328 |
| | PP | 0.590 | 0.244 | 0.345 | 0.071 | 0.217 | 4.422 | 0.486 |

[a]Species: CV—*Calicotome villosa*; CS—*Ceratonia siliqua*; QS—*Quercus calliprinos*; PP—*Pistacia palaestina*.
[b]Group parameters: RHL—the difference between the HD and LD readily degradable fractions; SHL—the difference between the HD and LD slowly degradable fractions; DHL—the ratio of mean degradability rate of the slowly degradable fraction of LD, to that of HD; IBL—the size of the independently bound fraction in LD.

than those of DM and CP, but DIF was lower in the DM than in NDF and CP. None of these differences was significant. The HD of PP was the highest and that of QC was the lowest, with those of CV and CS between them. These differences were also not significant, mainly because of the large variation among components within species. The differences among species in LD were also not significant, but the differences of DIF among species were highly significant ($P = 0.006$), the DIF values being positively related to the tannin content of the foliage.

Regression analysis for the dependence of DIF on either the tannin or the total phenol content of the foliage indicated that non-linear regression could describe this dependence better then linear regression in both cases. The percentage of the variance accounted for by the regression of the dependence of DIF on tannin content increased from 61.2 to 71.8 when quadratic rather then linear relations were assumed. The constant of this regression, however, was estimated to be 0.89, which leads to a predicted 8.9% of suppression of degradability when no tannins are present. The best predictor of DIF according to total phenols content was the exponential regression, for which the percentage of the variance accounted for by the regression increased from 59.2 to 74.0, compared with linear regression. This regression predicts no suppression of degradability at a total phenol content of 0.3% (Fig. 4).

The dependence of $E$ on the group parameters RHL, SHL, DHL, and IBL is presented in Table 4. These group parameters represent differences or ratios between HD and LD parameters; they are, therefore, considered to relate to the tannin content of

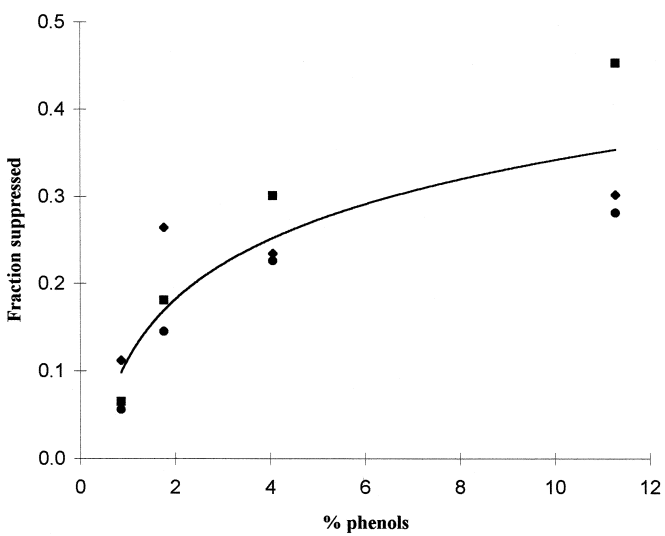


Fig. 4. The dependence of DIF, the suppressive effect of tannins on the effective degradability ($E$), on the percentage of total phenolic in the sample. This suppressive effect was expressed by the difference in $E$ between incubations in the presence (HD) and absence (LD) of PEG. The fraction suppressed was estimated for the DM (●), NDF (■) and CP (♦) components in the foliage of four species of trees, differing in their condensed tannins and total phenolic contents. The presented relation follows the exponential equation: $Y = 0.0995 * \ln(X) + 0.113$; $r^2 = 0.763$.

Table 4
Single and multiple linear regressions: probability ($P$) for the effects of several group parameters[a] of model 3 on the effective degradability ($E$) estimated by the high degradability (HD) or low degradability (LD) curves, or the difference (DIF) between these $E$ values. Percentage accounted for by the regression is also presented

| Group parameter | $P$ values | | | | | Percentage accounted for |
|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | General | |
| *HD*: | | | | | | |
| RHL | | | | | 0.557 | – |
| SHL | | | | | 0.638 | – |
| DHL | | | | | 0.537 | – |
| IBL | | | | | 0.978 | – |
| RHL + SHL + DHL + IBL | | | | | 0.915 | – |
| *LD*: | | | | | | |
| RHL | | | | | 0.228 | 5.6 |
| SHL | | | | | 0.805 | – |
| DHL | | | | | 0.382 | – |
| IBL | | | | | 0.268 | 3.3 |
| RHL + SHL + DHL + IBL | | | | | 0.360 | 9.5 |
| *DIF*: | | | | | | |
| RHL | | | | | 0.249 | 4.3 |
| SHL | | | | | 0.897 | – |
| DHL | | | | | 0.030 | 32.9 |
| IBL | | | | | 0.090 | 18.6 |
| DHL + IBL | 0.015 | 0.039 | | | 0.012 | 54.6 |
| SHL + DHL + IBL | 0.014 | 0.004 | 0.002 | | 0.002 | 77.0 |
| RHL + SHL + DHL + IBL | 0.987 | 0.129 | 0.011 | 0.008 | 0.008 | 73.7 |

[a]Group parameters: RHL—the difference between the HD and LD readily degradable fractions; SHL—the difference between the HD and LD slowly degradable fractions; DHL—the ratio of mean degradability rate of the slowly degradable fraction of LD, to that of HD; IBL—the size of the independently bound fraction in LD.

the tested foliage. The $E$ of both HD and LD was not associated with any of these group parameters, as expected. The DIF was positively associated ($P = 0.03$) with DHL, which represents the decrease in the degradation rate of the slowly degradable fraction, attributed to the tannin effect. It was also tended ($P = 0.09$) to be positively associated wit IBL, which represents the fraction independently bound to tannins. Multiple regression analysis of DIF against these two parameters showed a significant dependence, which accounted for 54.6% of the total variance. When SHL, was added to the regression, the percentage of the total variance which was accounted for by the equation increased to 77%, and addition of RHL to the equation did not yield further increase in the percentage of total variance accounted for by the regression.

## 4. Discussion

The degradation rate parameters and effective degradability of DM, fibre components and CP were investigated for many feedstuffs, using the Ørskov and McDonald (1979)

Model (e.g., Mertens and Loften, 1980; Petit and Tremblay, 1992; Andrighetto et al., 1993). The lag time parameter *L* was ignored in some of these studies, and was included as a discrete (Mertens and Loften, 1980) or continuous (Andrighetto et al., 1993) parameter. In the present study *L* was significant in some of the data sets in both the HD and LD curves (see for example Fig. 3d), suggesting that when low degradability feedstuffs are concerned, lag time should be always considered.

Model 1 parameters could not be well fitted to the observed data of material disappearance from dacron bags in the absence of PEG (the LD curve). This failure was due both to the inability of the model to follow the accumulation of material in the bags in the first 12–24 h ('negative degradability'), and to the change with time in the rate of disappearance thereafter. As a result, the mean SS of the LD curve in Model 1 was 7.7 times larger than that of the same curve in Model 3 (Table 2). On the other hand, the fit of the HD curve by Model 1 was quite good, although in some cases, especially for the NDF component, this fit could be improved either by modifying the degradability rate with time, as suggested by France et al. (1993), or by dividing the slowly degradable material into two separate pools, degraded at different rates, as was done in Model 3 in the present study. It could be observed from the comparison between the HD curves of the PP NDF component obtained by fitting outputs from Model 1 and Model 3, respectively (Fig. 3), that the addition of this division in Model 3 resulted in a considerable improvement of the fit. Model 2 failed to fit either the HD or the LD curve, with mean SS values 4.6 and 4.3 times larger, respectively, than those of Model 3 (Table 2). This was because Model 2 was based on the assumption that the kinetic parameters are not altered by the tannins, and that the difference between the HD and LD curves could be fully explained by the free-material binding capacity of tannins, so that the HD parameters were not independent of the LD curve, and they were affected by the LD observations. As a result, both fits were not good. Model 3 allowed for the effects of tannin on the kinetic parameters of the degradation, as well as a capacity of tannins to bind free materials. The fit of this Model to both HD and LD data was significantly better than those of Models 1 and 2, suggesting that both effects of tannins are active. The existence of cellulase inhibitors in tannins is indicated by Van Soest (1994), based on studies reported by Mandels and Reese (1963), and Robbins et al. (1975). Inhibition of cellulose degradation in vitro by tannin-containing extract was reported also by Tagari et al. (1965). In the present study, Model 3 estimates lower degradation rates for NDF as well as for CP and DM components, suggesting that not only cellulose degradation is affected. The capacity of tannins to form complexes with soluble matter, especially protein, in the rumen environment is well established in many studies (e.g. Kumar and Vaithiyanathan, 1990). However, whether the observed reduction (e.g. in the study of Robbins et al., 1975) in digestibility was a result of specific inhibition of degradation or unspecific precipitation is not known (Van Soest, 1994). We suggest that Model 3 in the present study offers a possibility to differentiate these two processes. According to this model estimations, both processes take place in both cell wall and protein components.

No relationship to the tannin content was found for the *E* of HD for any of the components (Table 3), suggesting that 2 g of PEG, when incorporated in the 5 g of material in the dacron bags, could prevent most, if not all, of the effect of tannin on

degradation. The *E* of LD was also not significantly related to the tannin content, probably because of the wide variations among species and among components in the *E* of HD. This variation was reflected in the *E* of LD, because the effect of tannins was defined in terms of the difference between the two curves. This difference in *E* between HD and LD was significantly affected by the tannin content. The general trend of increasing tannin effect with increasing tannin content (Fig. 4) could be observed for all the material components. However, although tannins have been reported to bind proteins primarily (Jones and Mangan, 1977; Kumar and Vaithiyanathan, 1990), the mean decrease in *E* attributed to the tannin effect in the present study was similar for the CP and NDF components, and only slightly larger than that for the DM, with no significant differences among components. This was probably due in part to the free material which was bound to tannins, assuming that a considerable part of it was not removed by the neutral detergent digestion. In the calculations this latter part was treated similarly to the original NDF, leading to an increased estimate of the effect of tannin on its degradability.

The dependence of the tannin effect on tannin content, however, was not linear, and the quadratic regression predicted a considerable effect on degradability of approximately 9% with zero tannin content. The constant value became more acceptable when total phenols, rather than condensed tannins, were considered as the independent parameter (Fig. 4). The content of condensed tannins found in a given material depends on the method of extraction (Hagerman, 1988; Silanikove et al., 1994); the method used in the present study, gave almost zero content of condensed tannins in CV, but the estimated effect was still considerable. On the other side of the range, the condensed tannin content of PP was more than 3 times as great as that of QC, but its effect was only moderately larger. It is suggested that the condensed tannin content does not necessarily provide the best prediction of the effect of tannin on degradability, and that relations of the decrease in degradability due to tannins should be studied with reference to better defined components of the polyphenol complex. In the present study, the content of total phenols could provide as good a prediction of the tannin effect as the condensed tannin content, and with a more acceptable zero-point value. The relationships between the effect of tannin on degradability and several group parameters, derived from the parameters of Model 3 were examined. It was found that *E* of HD and of LD were not associated with any of these group parameters and, when tested separately, only two of the four group parameters were associated with DIF, which was defined to reflect the tannin effect on degradability (Table 4). The two group parameters that relate to the difference between the readily and slowly degraded pools in the presence (HD) and absence (LD) of PEG were not associated with the effect of tannins. However, in a multiple regression test it was shown that three out of the four group parameters could account for 77% of the total variance of DIF. The change in the rate of degradation in the present study was significantly associated with the tannin effect, suggesting that the contribution of tannins to the decrease in *E* was more a result of a delay in digestibility than reduction of the potentially degraded fractions. Another part of the effect was a result of the free-material binding capacity of the tannins, represented by the group parameter, IBL. Because this bound material was indicated by Model 3 to be subsequently released, but at a very slow rate, it is suggested that the result of the

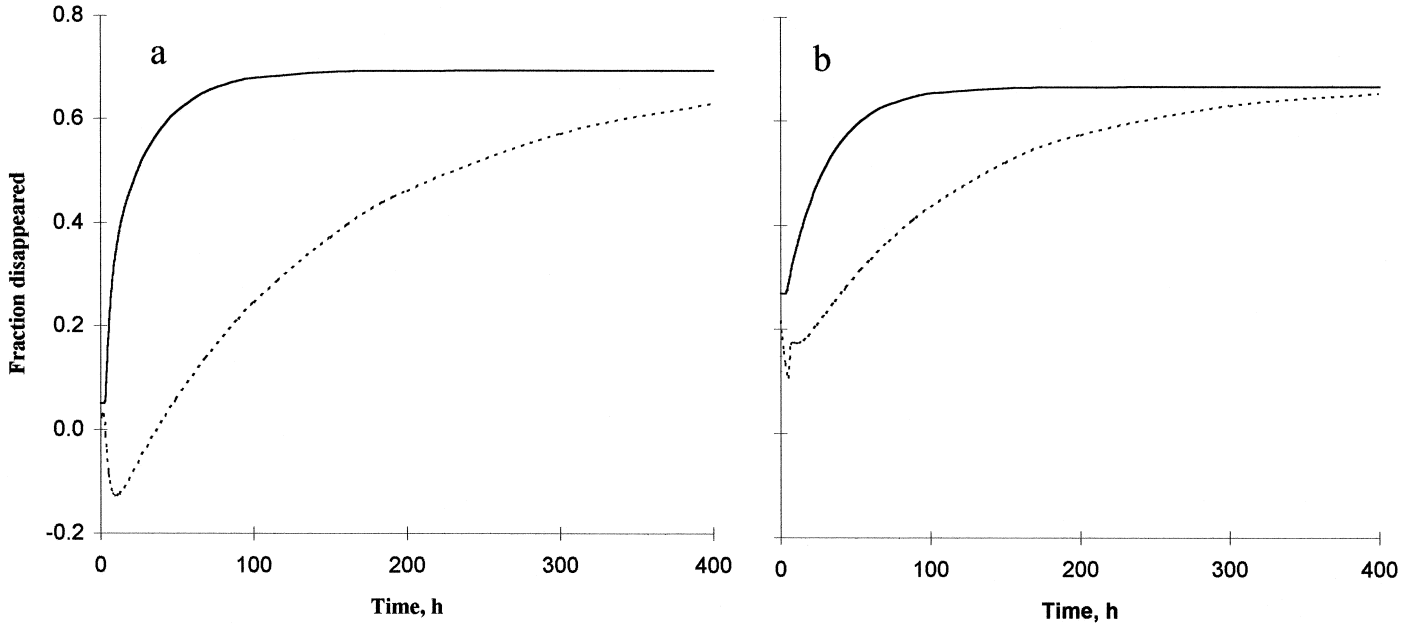


Fig. 5. Extrapolation of the estimated degradation in the presence (HD, solid line) and absence (LD, dotted line) of PEG, to an incubation time of 400 h. (a) NDF component of *Pistacia palaestina*; (b) DM component of *Quercus calliprinos*.

existence of this pool is also a delay in degradation rather than reduction in the potentially degraded pool. When the calculated HD and LD curves were extrapolated to an incubation period of 400 h (Fig. 5), it could be observed that the estimated accumulated degradations with and without PEG were almost the same by the end of the 400-h period.

## 5. Conclusions

The tannin effect could be evaluated according to the difference between the estimates of $E$ in the presence or absence of PEG, assuming that PEG totally prevents the effect of tannins on the kinetics of in situ degradation. The tannin effect which was estimated in this way showed a non-linear dependence on the tannin or total phenol content of the tested material. This effect could be explained in terms of a combination of a reduction in the rate of degradation of potentially degraded material, and of a capacity of tannin to bind and hold free material. The tannin effect was not related to the content of non-degradable material.

## Acknowledgements

Contribution from the Agricultural Research Organization, the Volcani Center, Bet Dagan, Israel No. 1976-E, 1996 series.

## References

Aharoni, Y., Tagari, H., Boston, R.C., 1991. A new approach to the quantitative estimation of metabolic pathways in the rumen. Brit. J. Nutr. 66, 407–422.

Andrighetto, I., Bailoni, L., Cozzi, G., Tolosa, H.F., Hartman, B., Hinds, M., Sapienza, D., 1993. Observations on in situ degradation of forage cell components in alfalfa and Italian ryegrass. J. Dairy Sci. 76, 2624–2631.

AOAC. Official Methods of Analysis of The Association of Official Analytical Chemists. 14th edn. 1984. In: Williams, S. (Ed.). AOAC: Arlington, VA.

Berman, M., Shahn, E., Weiss, M.F., 1962. The routine fitting of kinetic data to models: a mathematical formalism for digital computers. Biophys. J. 2, 275–287.

Boston, R.C., Greif, P.C., Berman, M., 1982. CONSAM (conversational version of SAAM modelling program). In: Berman, M., Grundy, S.M., Howard, B.V. (Eds.), Lipoprotein Kinetics and Modelling. Academic Press. New York.

France, J., Dhanoa, M.S., Theodorou, M.K., Lister, S.J., Davies, D.R., Isac, D., 1993. A model to interpret gas accumulation profiles associated with in vitro degradation of ruminant feeds. J. Theor. Biol. 163, 99–111.

Hagerman, A.E., 1988. Extraction of tannin from fresh and preserved leaves. J. Chem. Ecol. 14, 453–461.

Garrido, A., Gomez-Cabrera, A., Guerrero, J.E., van der Meer, J.M., 1991. Effects of treatment with polyvinylpyrrolidone and polyethylene glycol on faba bean tannins. Anim. Feed Sci. Technol. 35, 199–203.

Jones, W.T., Mangan, J.L., 1977. Complexes of the condensed tannins of sainfoin (*Onobrychis viciaefolia Spp*) with fraction 1 leaf protein and with submaxillary mucoprotein and their reversal by PEG and pH. J. Sci. Food Agric. 28, 126–136.

Kumar, S., Vaithiyanathan, S., 1990. Occurrence, nutritional significance and effect on animal productivity of tannins in tree leaves. Anim. Feed Sci. Technol. 30, 21–38.

Mandels, M., Reese, E.T., 1963. Inhibition of cellulases and beta-glucosidases. Advances in Enzymatic Hydrolysis of Cellulose and Related Materials. In: Reese, E.T. (Ed.), McMillan, New York. pp. 115–158.

Mertens, D.R., Loften, J.R., 1980. The effect of starch on forage fiber digestion kinetics in vitro. J. Dairy Sci. 63, 1437–1446.

Ørskov, E.R., McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. Camb. 92, 499–503.

Petit, H.V., Tremblay, G.F., 1992. In situ degradability of fresh grass conserved under different methods. J. Dairy Sci. 75, 774–781.

Porter, L.J., Hrstich, L.N., Chan, B.C., 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry 25, 223–230.

Pritchard, P.H., Martin, P.R., O'Rourke, P.K., 1992. The role of condensed tannins in the nutritional value of mulga (*Acacia aneura*) for sheep. Aust. J. Agric. Sci. 43, 1739–1756.

Robbins, C.T., Van Soest, P.J., Mautz, W.W., Moen, E.N., 1975. Feed analysis and digestion with reference to white-tailed deer. J. Wildl. Manage. 39, 67–79.

Setala, J., 1983. The nylon bag technique in determination of ruminal feed protein degradation. J. Sci. Agric. Soc., Finland 55, 1–78.

Silanikove, N., Nitsan, Z., Perevolotsky, A., 1994. Effect of daily supplementation of polyethylene glycol on intake and digestion of tannin-containing leaves (*Ceratonia siliqua*) by sheep. J. Agric. Food Chem. 42, 2844–2847.

Silanikove, N., Shinder, D., Gilboa, N., Nitsan, Z., 1996. Binding of poly (ethylene glycol) to samples of forage plants as an assay of tannins ans their negative effects on ruminal degradation. J. Agric. Food Chem. 44, 3230–3234.

Swain, T., Hillis, W.E., 1959. The phenolic constituents of Prunus domestica: I. The quantitative analysis of phenolic constituents. J. Sci. Food Agric. 10, 63–68.

Tagari, H., Henis, Y., Tamir, M., Volcani, R., 1965. Effect of carob pod extract on cellulolysis, proteolysis, deamination, and protein biosynthesis in an artificial rumen. Appl. Microbiol. 13, 437–442.

Terril, T.H., Douglas, G.B., Foote, A.G., Purchas, R.W., Wilson, G.F., Barry, T.N., 1992. Effect of condensed tannins upon body growth, wool growth and rumen metabolism in sheep grazing sula (*Hedysarum coronarium*) and perennial pasture. J. Agric. Sci. 119, 265–274.

Van Soest, P.J., 1994. Nutrition Ecology of the Ruminant (2nd edn.). Cornell University Press, Ithaca, NY, pp. 196–212.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods of dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.

Waldo, D.R., Smith, L.W., 1972. Model of cellulose disappearance from the rumen. J. Dairy Sci. 55, 125–129.