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Correcting the Calculation of Extent of Degradation to Account for Particulate Matter Loss at Zero Time When Applying the Polyester Bag Method

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ABSTRACT: The established calculation of ruminal extent of degradation using the polyester bag method overestimates extent. The wash fraction, at least in part, is subject to losses from the rumen due to passage. Four formulae are proposed to minimize this risk of overestimation. Four options are considered: 1) passage losses for particulate matter escaping from the bag at zero time are according to the particulate fractional passage rate, 2) the liquid rate, and 3) the average

of the two, and additionally 4) there is no instantly degradable fraction. The established and proposed formulae were examined using polyester bag data for six forages: grass silage, fresh ryegrass, fresh white clover, alfalfa hay, mixed grasses hay, and hay from a permanent mountain meadow. The established formula gave appreciably higher estimates of extent of degradation in all cases. Overestimation was at least 6.9, 4.9, 2.9, 2.1, 2.4, and 4.5%, respectively, for the six forages.

Key Words: Rumen Digestion, Ruminant Feeding, Mathematical Models, Dry Matter Digestion

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Introduction

Extent of degradation of ruminant feeds has been determined for most of this century, mainly using the polyester bag method (Quin et al., 1938; Van Keuren and Heinemann, 1962; Ørskov and McDonald, 1979). The main purpose of placing a feed sample inside the bag suspended in the rumen of an animal is to eliminate the effects of passage or outflow and measure losses due to degradation alone. Some of the undegraded feed inevitably escapes from the bag. A portion of the insoluble fraction, over and above the truly soluble fraction, escapes at zero time (fraction β) and forms part of the intercept for the degradation curve (Figure 1). For increasing values of β , the curve will be displaced higher and higher on the y-axis, yet the calculated fractional rate of degradation will be much the same (López et al., 1995). The fractional rate is unlikely to be exactly the same because the feed remaining in the bag contains more of the larger particles, offering a reduced surface area for microbial attachment.

No control can be exercised over the escaped fractions experimentally, and this rules out any direct measurements of the β fraction, even if a marked or mordanted feed sample is used. The fraction washed out of the bag at zero time is (improperly) called the soluble fraction because the polyester bag method relies on the assumption that the escaped fraction is completely and rapidly degraded (i.e., disappearance is synonymous with degradation). It has been virtually impossible to test this assumption in vivo. In our view, the problem has been compounded further by the introduction of washing machines in place of gentle hand-washing under running tap water. Centrifugal and other mechanical forces drive even more of the insoluble fraction out of the bag. The proportion of dry matter loss using a washing machine was .31 and .48, compared to the corresponding estimate by hand-washing of .16 and .14 for grass and maize silage, respectively (Cockburn et al., 1993). The corresponding dry matter solubility measured using filter paper was found to be .12 and .10 for the two silages. This overestimation of the soluble fraction leads to a positive bias when calculating extent of degradation in the rumen; the bias has ranged from 4 to 38%. In this article, four formulae for correcting the extent of degradation calculation to account for the bias are proposed and evaluated using a range of forages.

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Materials and Methods

The main components of a sample of idealized substrate undergoing degradation in situ are illustrated in Figure 2. The substrate is fractionated (units are in grams per gram of feed sample incubated) as:

1. A truly soluble fraction S that is degraded instantly and completely.
2. An escaped insoluble fraction β , consisting of mainly small particles, that is degraded at a rate in the range shown (rates shown are m , $2m$, $5m$, $10m$, and $20m$, with m set arbitrarily equal to .035 per hour), leaving behind an undegradable fraction. Thus β comprises two subfractions: β_D , which can be degraded over time, and β_U , which is undegradable (i.e., $\beta = \beta_D + \beta_U$).
3. An insoluble fraction B that does not escape and degrades in the bag at a rate c (per hour).
4. A completely undegradable residue U that remains in the bag.

In applying the polyester bag method, the time course of cumulative degradation of substrate is generally described using a simple Mitscherlich model (Ørskov and McDonald, 1979; McDonald, 1981; Dhanoa, 1988). Mathematically, the model is summarized as follows:

$$y_t = A + B(1 - e^{-c(t-T)}), t \geq T, \quad [1]$$

where y_t (grams lost/grams incubated) is disappearance to time t (hour), and A , B , c , and T are constants. A

represents the so-called soluble fraction (assumed instantly degraded), B the degradable part of the insoluble fraction, c the fractional degradation rate, and T (hour) the lag time before the commencement of degradation of B . A includes both the truly soluble fraction and the escaped insoluble fraction (i.e., $A = S + \beta$). Estimates of the parameters A , B , c , and T , used in conjunction with an estimate of k_p (per hour), the ruminal rate of passage, permit evaluation of the extent of ruminal degradation E (grams degraded/grams ingested) by applying the following formula:

$$E = A + Bce^{-k_p T}/(c + k_p). \quad [2]$$

The formula assumes that there is no lag associated with passage.

This classical analysis, however, ignores the fraction β . It assumes that all of fraction A disappears instantly, and none of it is subject to losses by passage; it assumes $A \equiv S$. In the rumen, this assumption is valid for the truly soluble fraction S , but β_D must be degraded over time at a certain rate, and, therefore, some of it will flow out of the rumen before being degraded. Taking this into account, the corrected formula for E is:

$$E_I = S + Dce^{-k_p T}/(c + k_p), \quad [3]$$

where E_I denotes corrected E , and S and D are the truly soluble and the insoluble but potentially degradable

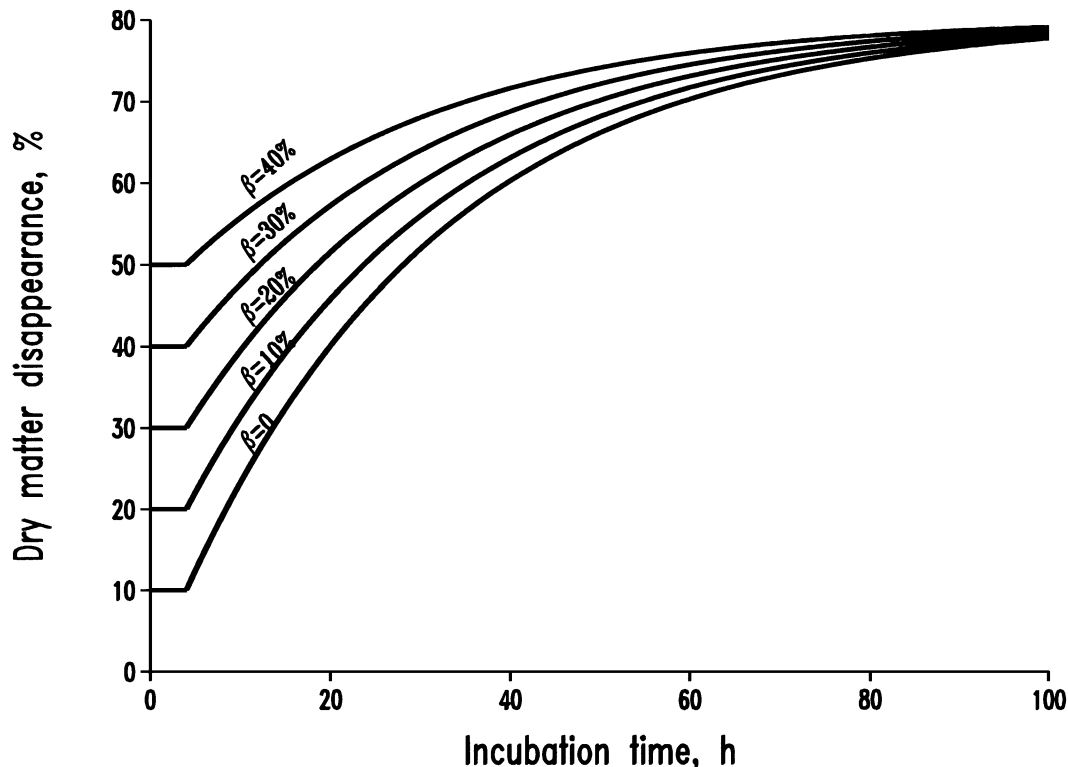


Figure 1. Influence of the escaped insoluble fraction β on a typical degradation curve.

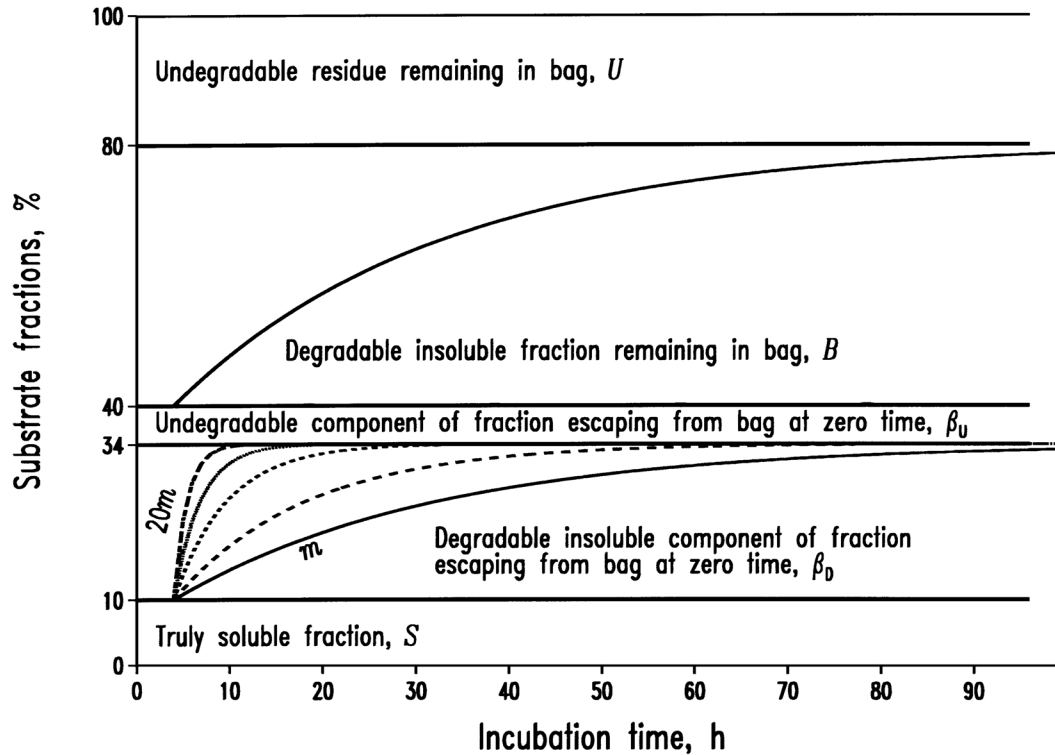


Figure 2. Components of an idealized substrate undergoing degradation in situ (see text for details).

fractions, respectively. Assuming the same degradation rate for B and β_D , Eq. [3] can be expressed as:

$$E_I = (A - \beta) + (B + \beta_D)ce^{-k_p T}/(c + k_p), \quad [4]$$

which gives:

$$E_I = E - \beta_U - \beta_D[1 - ce^{-k_p T}/(c + k_p)]. \quad [5]$$

Thus $E > E_I$, and the size of the error in calculating extent of degradation is affected by the relative sizes of c and k_p . To apply the suggested correction, fractionation of β into β_D and β_U is required. This is achieved by assuming that the degradable and undegradable fractions as a proportion of the insoluble fraction are the same in the escaped fraction β and in the fraction remaining in the bag, resulting in:

$$\beta_D = \beta B/(1 - A), \quad [6]$$

and

$$\beta_U = \beta(1 - A - B)/(1 - A). \quad [7]$$

If, as in Cockburn et al. (1993), a measure of the truly soluble fraction is available, β can be determined as $A - S$, β_D and β_U can then be calculated, and the overestimation error in E can be rectified using Eq. [5]. Eq. [5] can be simplified to give:

$$E_I = [E(1 - S) - \beta]/(1 - A). \quad [8]$$

This is equivalent to the correction formula proposed by Lopez et al. (1994) and France et al. (1997); the equivalence can be shown by putting $\lambda [= \Delta_0/(1 - y_0^{(c)})]$ equal to $\beta/(1 - S)$.

The problem regarding the escaped fraction β is more complicated than discussed above. The relevant fractional rate of passage for β may well be that for the liquid, k_l (per hour), rather than that for the particulate matter, k_p , which is more appropriate for the fraction remaining in the bag. Toward this end, the following options are proposed and the extent of degradation calculated accordingly, depending on one's choice of assumptions:

1. Passage losses for the β fraction are according to the liquid fractional passage rate k_l , giving:

$$E_2 = (A - \beta) + \beta_D ce^{-k_l T}/(c + k_l) + B ce^{-k_p T}/(c + k_p). \quad [9]$$

2. In the absence of reliable evidence, passage losses for β are assumed to be the average of k_l and k_p , giving:

$$E_3 = (A - \beta) + \beta_D ce^{-(.5k_l + .5k_p)T}/(c + .5k_l + .5k_p) + B ce^{-k_p T}/(c + k_p). \quad [10]$$

3. Partitioning of the incubated substrate disappearing from the bag at zero time is avoided, and it is assumed the fraction remaining in the bag is representative of the substrate as a whole (there is no instantly degradable fraction). Thus, both substrate fractions (i.e., A and B) are assumed to

degrade at the same rate and to the same extent, giving:

$$E_d = (A + B)ce^{-k_p T}/(c + k_p). \quad [11]$$

Disappearance curves were obtained for six forages: grass silage, fresh ryegrass, fresh white clover, alfalfa hay, mixed grass hay, and hay from a permanent mountain meadow. The silage and fresh forages were freeze-dried and ground for incubation in polyester bags and for solubility determinations. The truly soluble fraction S was measured after soaking a 1-g sample in 50 mL of distilled water at 39°C for 1 h with occasional shaking, and then filtering through Whatman no. 1 filter paper, repeatedly washed with distilled water. The wash fraction A was measured by washing polyester bags, containing samples of the forages, in a washing machine (short cycle with cold water). Degradation parameters were estimated by fitting the simple Mitscherlich model to the disappearance curves. Extent of degradation was estimated using Eq. [2], [4], [9], [10], and [11]. Eq. [2] represents the situation in which errors due to the escaped fraction β are ignored, whereas the other equations allow some correction, depending on the underlying assumptions. Details of the forage samples and the in situ procedure are given by López et al. (1995, 1998).

Results

Values for the extent of degradation in the rumen for the six forages calculated using the different formulae are shown in Table 1. Values of E determined without

correcting for particle losses at zero time were always higher than corrected values. The largest difference was observed using Eq. [11] for correcting, which assumes fractions A and B behave identically. This formula is likely to underestimate extent, because the truly soluble part of A (i.e., S) should be fermented at a faster rate than the insoluble fraction B (see Stefanon et al., 1996; Weisbjerg et al., 1998). The other formulae gave corrected values that varied over a small range within each forage type. The bias in relation to uncorrected E became larger as the fractional passage rate assumed for β_D was increased. The magnitude of this bias varied depending on the estimates of the degradation parameters. Therefore, a sensitivity analysis was undertaken using the correction represented by Eq. [5] to examine the effects of the values of the degradation parameters and the passage rate on the bias between corrected and uncorrected E .

Figure 3 shows the effect of overestimating the soluble fraction (increasing the β values) on the difference between uncorrected and corrected E . The relationship between both variables is linear, with a greater slope as the proportion of β_U , the undegradable matter, in the particulate matter loss is increased. The relationship between the degradation and passage parameters and the bias in calculating E is presented in Figure 4. The bias showed little variation over a wide range of T (lag time) values. However, the bias in E varied over a range of values of the degradation and passage rates. This bias decreased as the values of c were increased, and increased with higher values of k_p . Both relationships are curvilinear with an asymptote. There is competition between the processes of degradation and passage. If

Table 1. Extent of degradation in the rumen for six forages obtained using the different formulae and assuming different passage rates

Item	Grass silage	Fresh ryegrass	Fresh white clover	Alfalfa hay	Grass hay	Permanent meadow hay
Degradation parameters						
A (g/g incubated)	.388 (<.0001) ^a	.447 (<.0001)	.542 (<.0001)	.357 (.0030)	.354 (.0007)	.245 (.0011)
B (g/g incubated)	.458 (.0087)	.450 (.0105)	.408 (.0023)	.404 (.0076)	.517 (.0149)	.436 (.0094)
c (per h)	.037 (.0015)	.047 (.0022)	.106 (.0051)	.176 (.0161)	.062 (.0095)	.047 (.0027)
T (h)	5.1 (.26)	3.2 (.45)	3.5 (.32)	.8 (.48)	1.1 (.58)	.5 (.28)
S (g/g incubated)	.285	.362	.470	.314	.307	.177
Extent of degradation (g/g ingested) ^b						
E (Eq. 2)	.590 (.0047)	.682 (.0066)	.817 (.0031)	.687 (.0069)	.674 (.0041)	.495 (.0073)
E_1 (Eq. 4)	.521 (.0055)	.633 (.0076)	.788 (.0036)	.666 (.0060)	.650 (.0048)	.450 (.0087)
E_2 (Eq. 9)	.503 (.0052)	.617 (.0074)	.773 (.0039)	.662 (.0063)	.641 (.0048)	.442 (.0086)
E_3 (Eq. 10)	.509 (.0053)	.623 (.0075)	.779 (.0038)	.664 (.0062)	.645 (.0048)	.445 (.0087)
E_4 (Eq. 11)	.373 (.0068)	.469 (.0090)	.641 (.0092)	.622 (.0153)	.539 (.0110)	.391 (.0096)
Extent of degradation (g/g ingested) ^c						
E (Eq. 2)	.503 (.0036)	.596 (.0057)	.739 (.0050)	.634 (.0134)	.581 (.0035)	.419 (.0049)
E_1 (Eq. 4)	.420 (.0042)	.534 (.0066)	.699 (.0057)	.610 (.0128)	.551 (.0042)	.367 (.0062)
E_2 (Eq. 9)	.407 (.0039)	.520 (.0063)	.682 (.0058)	.604 (.0130)	.543 (.0039)	.359 (.0060)
E_3 (Eq. 10)	.411 (.0040)	.525 (.0064)	.688 (.0058)	.607 (.0130)	.546 (.0041)	.362 (.0061)
E_4 (Eq. 11)	.213 (.0057)	.297 (.0087)	.461 (.0130)	.523 (.0267)	.383 (.0105)	.272 (.0069)

^aValues in parentheses are standard errors.

^bCalculated assuming a fractional passage rate for particles (k_p) = .033 and a fractional passage rate for the liquid phase (k_l) = .080.

^cCalculated assuming k_p = .067 and k_l = .160.

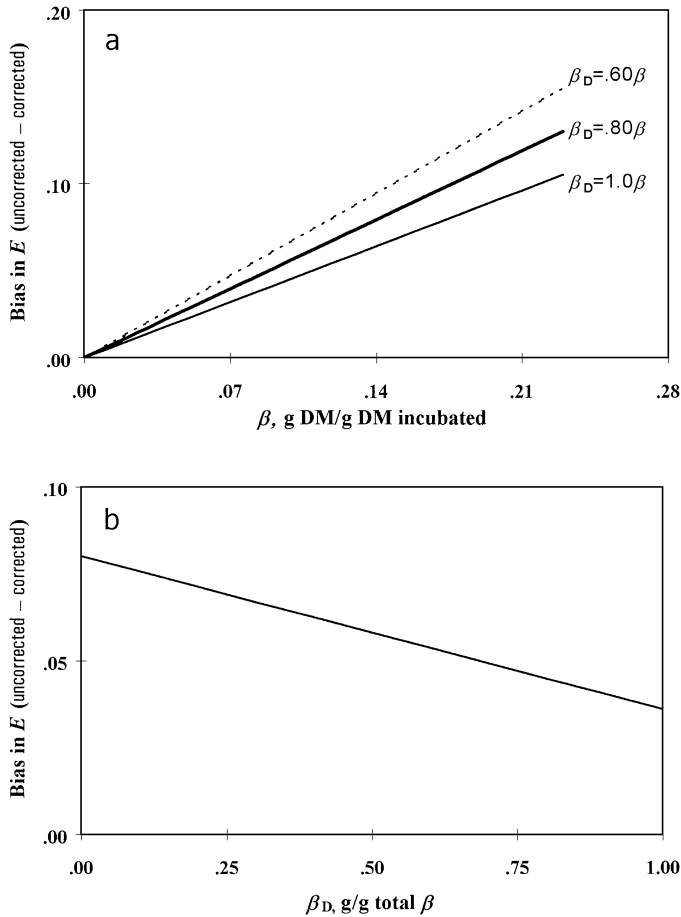


Figure 3. Effect of overestimating the soluble fraction by increasing (a) the escaped insoluble fraction β and (b) the degradable subfraction of β , β_D , on the extent of ruminal degradation E .

degradation prevails, more of the β_D fraction will be degraded in the rumen with smaller losses due to passage. In this situation, the bias between uncorrected and corrected values of E will be smaller. The graphs shown in Figures 3 and 4 are based on default parameter values of .28, .2, .576, .050, .033, and 3 for A , S , B , c , k_p , and T , respectively.

Discussion

Extractable components in water and buffer solutions vary from one feed to another and include minerals, protein, organic acids, simple sugars, and their short-chain polymers (Stefanon et al., 1996). Lister et al. (1992) conducted serial extraction from grasses with cold water, hot water, NDF, and ADF. The residue after each stage was evaluated using a near-infrared (NIR) spectrophotometer over the spectral range of 1,100 to 2,500 nm. After standardization of these spectra, difference spectra were calculated. The difference spectra gave qualitative assessment of the components that were lost at each stage of extraction. Cold water extraction removed fractions that have absorbancies in the

regions 1,628 nm (soluble sugars), 1,968 nm (soluble carbohydrates, nitrates, and nonprotein nitrogen), and 2,170 nm (soluble protein component corresponding to amide structures that are present in some proteins [Wetzel, 1983]). Thus the soluble part of an animal feed may well play an important role at the earlier stages of degradation by supporting optimal growth of microbial populations.

Most components of the soluble fraction will be degraded almost instantly in the rumen, probably due to their solubility in water. Although this fraction can outflow from the rumen at the same rate as the liquid, it has been accepted that these substrates are completely

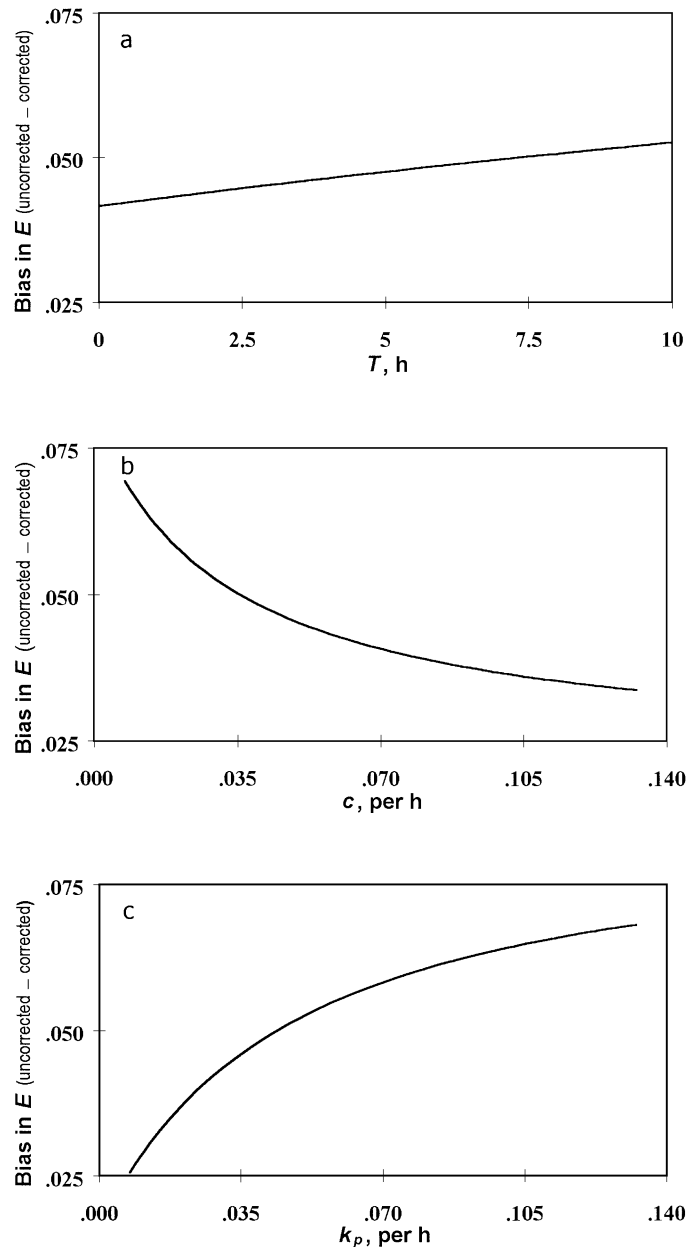


Figure 4. Relationship between the extent of degradation parameters (a) lag time T , (b) fractional degradation rate c , and (c) fractional passage rate k_p , and the bias in calculating the extent of ruminal degradation E .

fermented in the rumen because of their fast rates of degradation. Weisbjerg et al. (1998) obtained in vivo rates of hydrolysis of disaccharides of up to 14 h^{-1} for sucrose and 2.5 h^{-1} for lactose, and rates of fermentation of monosaccharides originating from disaccharide hydrolysis from 3 to 7 h^{-1} . With these rates of hydrolysis and fermentation, losses from passage are negligible, and, therefore, it can be assumed that the soluble fraction is completely and instantly degraded in the rumen, and the practical error introduced by this assumption can be considered insignificant. However, it is much less likely that the particulate matter lost from the bag at zero time is degraded at such fast rates, implying that some portion of the wash fraction leaves the rumen undegraded by outflow to the omasum. Therefore, models derived for estimating extent of degradation from kinetic data obtained with the polyester bag method should take into account that the wash fraction will also be subject to losses due to passage; otherwise extent of degradation in the rumen will be overestimated.

Four formulae (Eq. [4], [9], [10], and [11]) are presented for correcting the extent of degradation calculation (Eq. [2]) from the simple Mitscherlich model, to account for the heterogeneous nature of the wash fraction. The values obtained from these formulae are dependent on the rates of degradation and passage assumed for the degradable component of the escaped particulate matter. The formulae assume that the degradation rate of the insoluble but degradable component escaping from the bag undegraded is the same as that for the potentially degradable fraction remaining in the bag. This assumption is necessary because the polyester bag method does not allow any estimate of the former parameter to be obtained, although it can be speculated that there should be some difference between the degradation rates of the escaping and remaining fractions. The escaped fraction consists of smaller particles, which have been shown to be more extensively degraded mainly because fibrolytic ruminal bacteria degrade plant fibers by surface erosion and smaller particles occupy a larger surface area (France et al., 1993). However, the estimate of c for the degradable insoluble fraction remaining in the bag is inevitably somewhat lower than that for the whole sample because the faster-degrading fraction has escaped and plays no part in the estimation process. As a consequence, the passage loss adjustment factor $ce^{-k_p T}/(c + k_p)$ for a given value of k_p will be lower, thus cancelling some of the positive bias in the estimate of extent of degradation caused by assuming that the truly soluble fraction equals the wash value.

In applying the polyester bag method, the time course of cumulative degradation of substrate is generally described using a simple Mitscherlich model, and the corrective formulae presented herein are based on this model. The simple Mitscherlich model, however, only accommodates diminishing returns behavior. With forages and forage-based diets, for example, the degradation profiles sometimes exhibit sigmoidal rather than

diminishing returns behavior. Consequently we (Dhanao et al., 1995) proposed a new model to describe degradation in the rumen that was derived by postulating that the fractional degradation rate varies with time. Mathematically the model can be considered a generalization of the simple Mitscherlich and allows for underlying kinetics that are diminishing returns or sigmoidal in type. The extent of degradation calculation for this generalized Mitscherlich is more complicated, but the algebra can be expanded to accommodate all of the corrections for particulate matter escape at zero time that are proposed in this article for the simple Mitscherlich (Eq. [4], [9], [10], and [11]). Equally, the corrections can be incorporated into alternative models to the Mitscherlich (France et al., 1990; Dhanoa et al., 1996; López et al., 1999) for analyzing degradation profiles obtained when applying the polyester bag method, though the resultant algebra will likely be cumbersome.

Implications

The established calculation of ruminal extent of degradation using the polyester bag method overestimates extent. The wash fraction (assumed soluble) in fact comprises two subfractions of quite different kinetic behaviour (viz., the truly soluble fraction and the escaped particulate matter loss). This overestimation of the soluble fraction leads to a positive bias when calculating extent of degradation in the rumen. Four formulae may minimize this bias. Four options are considered: 1) passage losses for particulate matter escaping from the bag at zero time are according to the particulate fractional passage rate, 2) the liquid rate, and 3) the average of the two, and additionally 4) there is no instantly degradable fraction. Intuitively, Option 2 is to be preferred. This results in values for extent of ruminal degradation, and hence microbial protein supply at the duodenum, that are significantly smaller than those used in current feed evaluation systems.

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