ELSEVIER

Animal Feed Science and Technology
95 (2002) 93–104

ANIMAL FEED SCIENCE AND TECHNOLOGY

www.elsevier.com/locate/anifeedsci

# Partitioning of energy and degradability of browse plants in vitro and the implications of blocking the effects of tannin by the addition of polyethylene glycol

A.S.H. Baba[a,*], F.B. Castro[b], E.R. Ørskov[c]

[a]*Commonwealth Fellowship Grant, ISB, Universiti Malaya, 50603 Kuala Lumpur, Malaysia*
[b]*The BioSystem Group, R. Olavo Bilac, 288/83 Campinas, SP, Brazil*
[c]*Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK*

Received 15 June 2000; received in revised form 7 June 2001; accepted 19 July 2001

**Abstract**

The gas production in vitro method was used to evaluate the degradability and gas production of browse plants in the absence or presence of polyethylene glycol 8000 (PEG). Substrates (leguminous and browse plants; 500 mg) were incubated for 24 h and the accumulated gas produced recorded. The incubation contents of the syringes were transferred into nylon bags and the undegraded residues weighed after washing and drying to constant weight (syringe-nylon bag (SNB) method). Substrates were also incubated in the rumen in nylon bags for 24 h to determine in sacco degradability. Gas production ranged between 10.3 and 64.4 ml whereas dry matter degradation ranges between 27.3 and 70.9%. Addition of PEG, which minimised the inhibitory effects of tannin on microbial fermentation resulted in an increase in both gas production and degradability in vitro, which ranged from 25.7 to 64.2 ml and 34.2 to 75.0%, respectively. Correlation analysis of the DM degradability estimated by the SNB method and in sacco method was greater in the presence of PEG ($y = 0.71x + 14.9$; $r^2 = 0.92$) compared with absence of PEG ($y = 0.59x + 15.0$; $r^2 = 0.72$). Partitioning factor (PF) of substrate to gas, which was expressed as mg DM degraded/ml gas, reflects the variation in microbial biomass yield. The PF figures, which varied from 4.94–11.05 to $PF_{+PEG}$ values of 4.74–6.84 upon the addition of PEG, indicate the inhibitory effects of tannins on gas production. This suggests the presence of tannin has a potentially beneficial effect to protein nutrition of the host animal by altering partitioning of nutrients towards higher microbial yield rather than short chain fatty acids. PF values of browse plants determined both in the absence and presence of PEG may indicate the relative importance of

* Corresponding author. Tel.: +60-3-7967-4188; fax: +60-3-7967-4178.
*E-mail address*: ahmad@biology.um.edu.my (A.S.H. Baba).

0377-8401/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0377-8401(01)00283-8

tannins in different plant species on substrate degradability and partitioning of nutrients.
© 2002 Elsevier Science B.V. All rights reserved.

*Keywords:* Tropical browse plants; Tannin; Partition of nutrient; In vitro gas production; Polyethylene glycol

## 1. Introduction

The potential degradability and degradation rate of feeds can be estimated by the gas production in vitro method (Menke et al., 1979). It is based upon the measurement of gas produced, which is a result of short chain fatty acids (SCFA) and gases produced from substrate fermentation by the rumen microbes. This method was shown to be a reliable tool in feed evaluation because gas production has been well correlated with microbial protein synthesis (Krishnamoorthy et al., 1991), in vivo digestibility (Khazaal et al., 1993), and intake (Blümmel and Ørskov, 1993). Microbial biomass yield from substrate fermentation is the most important protein source for the host animal. The relationship between the SCFA produced in the rumen and microbial protein available to ruminants is known to be negative (Hungate, 1966). This has also been demonstrated in vivo (Leng, 1993) and in vitro (Blümmel et al., 1997a). This implies that feed samples should not only be evaluated based on gas production data because partition of energy released from degraded substrate contribute both to SCFA production and for microbial protein synthesis (Blümmel et al., 1994). The concept of partitioning of fermentation products (partitioning factor; PF) was introduced recently to express the conversion of energy from truly degraded substrate required to yield 1 ml of gas (Blümmel et al., 1997b). Truly degraded substrate is estimated by weighing the fermentation residue remaining after refluxing the fermented substrate with neutral detergent solution (Blümmel and Becker, 1997). PF value is unique to each substrate, and is not necessarily correlated to chemical composition. Plants with high PF values in general are highly digestible roughage and they correlated well with dry matter intake in ruminants (Blümmel et al., 1997b). Thus, assessment of PF values may be used as an important tool for feed evaluation.

The effect of tannins on PF values of browse plants has not been extensively investigated. This is largely due to the difficulty in quantifying the amount of substrate degraded in vitro. It is important to determine the PF values for browse plants because most of these plants contain condensed tannins, which may cause harmful effects on rumen microbial fermentation (Jones et al., 1994). When incubated in the presence of PEG, the gas produced from substrate fermentation increased (Khazaal et al., 1994; Makkar et al., 1995a,b) and this has been attributed to the binding of PEG to tannins, which are released during fermentation (Khazaal et al., 1993). Formation of PEG-tannin complexes has been suggested (Salawu et al., 1997) to prevent the accumulation of these compounds in the medium to a level which might be inhibitory to the microorganisms. Therefore, the degradability of browse plants in the presence of PEG is also expected to increase. Unfortunately, the analysis of true in vitro degradability (Blümmel and Becker, 1997) can be misleading in browse plants due to the presence of tannins (Makkar et al., 1995a,b) especially if particular tannins in browse plants do not limit in vivo digestion. This is because tannins form complexes with proteins (both from plants and microbial sources), which are largely insoluble in neutral detergent

solution, thus forming precipitates, which in turn will overestimate the undegradable fraction (Makkar et al., 1995a,b). Attempts to separate tannins from fermented substrate using a centrifugation method associated with detergent digestion were also reported. But, they give incorrect values because the centrifugal forces appeared to enhance the formation of tannin–protein complexes in the fibre (Makkar et al., 1998).

The aim of this study was to validate a method to estimate the effect of tannin on degradability and partitioning of nutrients of browse plant incubated in vitro.

## 2. Materials and methods

### 2.1. Substrates

Browses, leguminous plants and grasses (as described in Tables 1 and 2) were sampled from humid tropical climatic region (Malaysia, 2°30′N, 112°30′E). These were hand-harvested, air-dried in-doors for 5 days and milled in a hammer mill to pass through a 1.0 mm sieve.

### 2.2. Chemical analysis

Dry matter and ash contents were determined according to Association of Official Analytical Chemists (AOAC, 1990). All analytical data were corrected and expressed on

Table 1
Chemical composition of browse and leguminous plants collected from hot and humid tropical climate[a]

| Species | DM[b] | OM[c] | NDF[d] | ADF[e] | CP[f] | TETa[g] | TECTa[h] | TEPA[i] |
|---|---|---|---|---|---|---|---|---|
| *A. hispida* | 897 | 912 | 499 | 246 | 133 | 39.0 | 12.6 | 290.2 |
| *Acacia mangium* | 902 | 907 | 294 | 444 | 132 | 9.9 | 15.7 | 415.2 |
| *Artocarpus heterophylus* | 896 | 868 | 496 | 384 | 159 | 6.1 | 3.2 | 73.2 |
| *Bixa arellana* | 902 | 937 | 437 | 261 | 135 | 104.6 | 15.9 | 197.4 |
| *C. calothyrcus* | 898 | 948 | 737 | 698 | 110 | 16.7 | 18.5 | 88.5 |
| *C. odorata* | 887 | 890 | 365 | 356 | 208 | 25.8 | 7.5 | 34.4 |
| *C. kyllinga* | 906 | 885 | 455 | 330 | 101 | 47.8 | 7.5 | 72.1 |
| *L. leucocephala* | 921 | 910 | 318 | 215 | 243 | 14.8 | 39.8 | 264.8 |
| *M. esculanta* | 903 | 944 | 295 | 364 | 116 | 155.5 | 16.2 | 438.8 |
| *S. aromaticum* | 905 | 958 | 489 | 361 | 76 | 89.2 | 11.7 | 130.8 |
| *M. arabathricum* | 910 | 912 | 672 | 385 | 124 | 2.71 | 10.7 | 158.9 |

[a] Kuala Lumpur, Malaysia.
[b] Dry matter.
[c] Organic matter.
[d] Neutral detergent fibre.
[e] Acid detergent fibre.
[f] Crude protein. DM, OM, NDF, ADF CP expressed as g/kg DM.
[g] Total extractable tannin (tannic acid equivalent; mg/g DM).
[h] Total extractable condensed tannin (catechin equivalent; mg/g DM).
[i] Total extractable proanthocyanidins (absorbance at 550 nm/g DM); analysis was carried out in duplicate and coefficient of variation was less than 5%.

Table 2
Comparison of substrate degraded after in vitro incubation, estimated using NDS[a], SNB[b] and in sacco methods[c,d]

| Species | 24 h degraded substrate (%) | | | | S.E.D. |
|---|---|---|---|---|---|
| | GP24h | In vitro and 'washed' with NDS | In vitro and washed using nylon bag | In sacco | |
| Graminicae | | | | | |
| Ryegrass hay | 68.3 a | 54.2 x | 51.5 x | 52.1 x | 1.1 |
| Wheat straw | 44.5 b | 43.9 x | 38.3 y | 36.6 y | 1.4 |
| *P. purpureum* | 50.1 c | 55.5 x | 41.9 y | 48.7 z | 3.2 |
| *Paspalum* sp. | 51.2 c | 62.0 x | 62.7 x | 67.7 x | 4.6 |
| *Trypsacum* sp. | 45.2 b | 51.7 x | 40.6 y | 44.2 y | 1.8 |
| S.E.D. | 3.0 | | | | |

[a] NDS: neutral detergent solution.
[b] SNB: syringe-nylon bag.
[c] Means with different letters (x, y, z) in the same row for 24 h degraded substrate are different ($P < 0.05$).
[d] Means with different letters (a, b, c) for the gas production (GP24h) column are significant ($P < 0.05$)

DM basis. Neutral (NDF) and acid (ADF) detergent fibre analysis were carried out as described by Robertson and Van Soest (1981). Nitrogen (N) content in samples was determined by macro-N analysis (Foss Electric Ltd., UK) and N figures were multiplied by 6.25 to estimate crude protein (CP).

### 2.3. Extractable phenolic compounds

Analysis of phenolic compounds was carried out in four replicates as described by Khazaal et al. (1993). Total extractable tannin (TETa; as tannic acid equivalent) was calculated by the difference between total extractable phenolics (Julkunen-Tiito, 1985) and phenolic compounds remaining after absorption onto poly-vinylpolypyrrolidone (PPVP) (Makkar et al., 1992). Total extractable condensed tannins, measured as catechin equivalent, were determined by the vanillin assay (TECTa; Broadhurst and Jones, 1978) and expressed as absorbance read at 550 nm (TEPAs) using the proanthocyanidins assay (Porter et al., 1986).

### 2.4. Host animal diets and preparation of incubation medium

Sheep fitted with permanent rumen cannulae were used for in sacco degradation studies and as source of inocculum. Animals were fed at maintenance on diets consisting of 500 g/kg DM grass hay, 300 g/kg DM barley, 100 g/kg molasses, 91 g/kg DM fishmeal and 9.5 g/kg mineral mix and vitamin mix. The diet was fed twice a day at 08.30 and 16.00 h. Samples of rumen contents were collected from at least two sheep prior to morning feeding and transferred into pre-warmed $CO_2$-filled thermos bottles. Rumen fluid was strained through three layers of gauze under continuous flushing with $CO_2$ gas. The temperature of the rumen liquor was maintained between 37 and 39°C throughout the preparation of the incubation medium. Substantial changes in the medium composition suggested by Blümmel and Becker (1997) were adopted because the amount of substrate used in this

study increased from 200 to 500 mg. Buffer volume increased two-fold to increase the buffering capacity of the incubation medium. Equal amount of distilled water was added to match the increase in buffer volume so that the medium had the same osmolality as that for the original method.

### 2.5. In situ degradation

In situ degradation analysis was carried out as described by Ørskov and McDonald (1979) with the following changes; a feed sample (1.5 g, 1.0 mm) in triplicate was transferred into a pre-weighed nylon bag and the bag was tied with elastic band to allow a substrate: nylon bag surface area ratio of 5 mg/cm$^2$. The bags were incubated in the rumen for 24 h and subsequently washed in cold water for 20 min and dried at 65°C for 48 h. DM degraded in sacco was determined at 24 h because the values correlate well with voluntary feed intake (Blümmel and Ørskov, 1993). Subsequent estimation of DM degraded in vitro after 24 h incubation (see Section 2.7) may then be used for comparison with those determined in sacco.

### 2.6. In vitro gas production

Gas production was determined as described by Menke et al. (1979). Feed samples (500 mg) without or with 500 mg PEG 8000 (Sigma Chemical Co., UK) were incubated in 40 ml of incubation medium. Analyses were carried out in triplicate. Syringes were shaken twice up to 2 h fermentation, and once at every reading, which were taken after 2, 4, 6, 8, 12 and 24 h. Net gas production data were corrected for 500 mg substrate. A ryegrass grass hay was used as standard substrate to detect between-run effect. Incubation was terminated by immersing syringes in cold water and the degradability analysis was carried out immediately as described in the following section. Thus, in addition to the measurement of gas produced, the in vitro method also provide estimation of DM degraded which may then be compared with those estimated using the in sacco method.

### 2.7. Estimation of degraded substrate in vitro

Degraded substrate was estimated as the difference between the weight of substrate incubated and the weight of undegraded substrate following in vitro gas production determination (see Section 2.6). Undegraded substrate after 24 h incubation was determined using either NDF-digestion method (Van Soest and Robinson, 1985; Blümmel and Becker, 1997; NDS method) or nylon bag washing method (substrate incubated in syringes (Section 2.6) followed by washing with nylon bag; SNB method). The content of the syringe was completely transferred into a beaker by using 50 ml of NDF solution. The reaction mixture was refluxed for 1 h and the residue was filtered through a pre-weighed sintered glass crucible no. 2 (Gallenkemp, UK).

The SNB method was carried out by completely transferring the entire contents of the syringe into a pre-weighed nylon bag (8 cm × 16 cm; pore size 40–60 μm). The nylon bags were tied with rubber bands and then thoroughly washed. There was no difference ($P > 0.05$) in the effective nylon bag surface area (30, 50 and 75% of original surface

area) on the 24 h degradability data from *P. purpureum*. Therefore, 50% effective surface area (5 mg incubated sample/cm$^2$) was used for testing other samples.

### 2.8. Validation of SNB method

#### 2.8.1. Effect of catechin + PEG on gas production and degradability of straw

Browse plants contain tannins that may affect microbial fermentation of substrate. The effect of condensed tannin on fermentation of straw in vitro was investigated using the SNB method to demonstrate changes in wheat straw degradability as a result of addition of condensed tannin in the absence or presence of PEG. Catechin was added at 5 or 10% of the substrate weight in the absence or presence of equal amount of PEG.

### 2.9. Statistical analysis

Treatment effects on DM degraded (mg DM/24 h), gas produced (ml/24 h) and PF (mg DM/ml gas) were compared using the least significant difference method (Steele and Torrie, 1980). Differences in DM degraded, gas produced and PF were determined by a one-way analysis of variance, with treatment as source of variation in the model:

$$Y_{ij} = \mu + \alpha_i + E_{ij}$$

where $Y_{ij}$ is the dependent variable denoting the $j$th measurement from the $i$th treatment; $\mu$ the common mean; $\alpha_i$ the effect of the $i$th treatment, $i = 1$ and 2; and $E_{ij}$ is the random residual. Differences between treatments were considered statistically significant at the $P < 0.05$ level.

## 3. Results

The chemical composition data of browse and leguminous plants are presented in Table 1. There was a wide variation in crude protein (76–243 g/kg DM), NDF (294–499 g/kg DM) and ADF (215–698 g/kg DM) contents for browses and leguminous plants. The condensed tannins, as estimated by three different methods, i.e. TETa, TECTa and TEPA, ranged between 2.71 and 155.5 mg tannic acid equivalent/g DM, 3.2–39.8 mg catechin equivalent/g DM and 34.4–438.8 absorbance at 550 nm/g DM, respectively.

### 3.1. Estimation of degraded substrate: NDS digestion and nylon bag washing

Differences in the amount of degraded substrate in vitro or in sacco are presented in Table 2. Degradation was higher for ryegrass hay, *P. purpureum,* and *Trypsacum* sp. when these were determined by the NDS method and higher for *Paspalum* sp. when using the in sacco method. The SNB method tended to give the lowest values for degradation. When compared with the in sacco method it was found that the SNB method gave a higher correlation ($y = 1.06x + 0.29$; $r^2 = 0.98$) than the NDF method ($y = 0.90x + 2.88$; $r^2 = 0.68$). The SNB method was used in subsequent studies to estimate the degradation of browse plants.

Table 3
Influence of catechin ± PEG[a] on in vitro fermentation of straw and their effects on substrate degraded, gas production and PF[b,c]

| Treatment | ml Gas/24 h | % Substrate degraded/24 h | Partitioning factor (mg DM/ml gas) |
|---|---|---|---|
| Straw | 54.3 a | 39.6 a | 3.37 a |
| Straw + PEG | 55.3 a | 40.5 a | 3.35 a |
| Straw + 25 mg catechin | 50.5 a | 38.9 a | 3.55 a |
| Straw + 25 mg catechin + PEG | 54.3 a | 38.6 a | 3.29 a |
| Straw + 50 mg catechin | 38.1 b | 30.8 b | 3.77 a |
| Straw + 50 mg catechin + PEG | 47.9 c | 34.3 b | 3.31 a |
| S.E.D. | 4.5 | 3.7 | 0.24 |

[a] PEG: polyethylene glycol 8000.
[b] PF: partitioning factor.
[c] Means with different letters in the same column are different ($P < 0.05$).

### 3.2. Effect of condensed tannin on wheat straw degradation

The inhibitory effect of added catechin (25 mg or 50 mg) had a more pronounced effects on gas production (−6.9 and −29.8%, respectively) than on the effect on substrate degradation (−1.7 and −22.2%), although the difference was significant ($P < 0.05$) only at high levels of inclusion of catechin (Table 3). As expected, addition of PEG suppressed the inhibitory effect of catechin as expressed by gas production and substrate degradability. The removal of the inhibitory effect of catechin by PEG however was not complete, even though PEG itself has no significant effect ($P > 0.05$) on straw degradability in vitro. Addition of catechin increased marginally the PF values when compared with the control (3.77 and 3.37, respectively), but such an effect was not significant ($P > 0.05$).

In sacco degradability of browse plants was compared to in vitro DM degradability estimated using the SNB method either in the absence or presence of PEG (Table 4). The degradability in sacco ranged between 45.8 and 90.5%. The degradability in vitro using the SNB method was lower and varied between 27.3 and 70.9%. However, upon addition of PEG, degradability was positively affected, i.e. an increase between 1.7 and 34.4%. The raise in degradability of browse plants due to the inclusion of PEG was positively correlated with changes in gas production, which ranged from +1.9 to +149.5%. The largest increase was observed for *Caliandra calothyrcus* (149.5%) followed by *Manihot esculanta* (130.2%), *Acalipha hispida* (98.5%), *Syzygium aromaticum* (55.3%) and *Cyperus kyllinga* (37.1%). The addition of PEG resulted in the reduction of PF values, which ranged between −0.8 and −92.3%. The greatest reduction in PF values due to PEG, −92.3 and −84.8%, were observed for *M. esculanta* and *C. calothyrcus*, respectively, which also had the highest PF values (11.04 and 11.05, respectively) in the absence of PEG.

*Leucaena leucocephala* responded differently to PEG; an increase in gas production (+50.5%) was associated with very small (+1.7%) and no significant ($P > 0.05$) increase in dry matter degradability. Two plant species, *Chromolaena odorata* and *Melastoma*

Table 4
Fermentation of browse and leguminous plants in vitro in the absence or presence of PEG[a] and their effects on gas production, degraded substrate and PF[b,c]

| Plant sample & treatment | % DM degraded in sacco/24 h | % DM degraded in vitro/24 h | Volume of accumulated gas (ml)/24 h | Partitioning factor (mg DM/ml gas) |
|---|---|---|---|---|
| *M. esculanta* | 68.7 | 46.7 | 19.2 | 11.04 |
| *M. esculanta* + PEG | | 54.0 | 44.2 | 5.74 |
| *C. calothrcus* | 29.7 | 27.3 | 10.3 | 11.05 |
| *C. calothyrcus* + PEG | | 34.2 | 25.7 | 5.98 |
| *A. hispida* | 85.7 | 55.8 | 27.0 | 8.94 |
| *A. hispida* + PEG | | 75.0 | 53.6 | 6.32 |
| *S. aromaticum* | 57.3 | 47.1 | 32.0 | 6.85 |
| *S. aromaticum* + PEG | | 54.7 | 49.7 | 4.95 |
| *L. leucocephala* | 79.8 | 71.7 | 41.8 | 6.03 |
| *L. leucocephala* + PEG | | 72.9 | 62.9 | 5.25 |
| *A. mangium* | 53.2 | 50.1 | 31.6 | 7.49 |
| *A. mangium* + PEG | | 51.9 | 35.4 | 6.84 |
| *B. arellana* | 47.9 | 44.4 | 28.2 | 7.09 |
| *B. arellana* + PEG | | 47.9 | 33.7 | 6.52 |
| *M. marabathricum* | 45.8 | 48.4 | 45.1 | 5.07 |
| *M. marabathricum* + PEG | | 51.0 | 48.9 | 4.77 |
| *A. heterophylus* | 73.0 | 54.3 | 64.4 | 5.05 |
| *A. heterophylus* + PEG | | 71.5 | 64.2 | 4.74 |
| *C. kyllinga* | 49.7 | 38.8 | 35.6 | 4.94 |
| *C. kyllinga* + PEG | | 50.9 | 48.8 | 4.81 |
| *C. odorata* | 90.5 | 70.9 | 52.6 | 6.10 |
| *C. odorata* + PEG | | 74.7 | 53.6 | 6.05 |
| S.E.D. | 2.4 | 6.1 | 5.2 | 1.24 |

[a] PEG: polyethylene glycol 8000.
[b] PF: partitioning factor.
[c] Data are means of triplicate analyses.

*arabathricum* showed no significant ($P > 0.05$) response in gas and dry matter degradability upon addition of PEG. No relationship was found between the changes in substrate degradability or gas production, upon the addition of PEG, and TETa, TECTa or TEPA.

## 4. Discussion

### *4.1. Estimation of in vitro degradability measured through nylon bag*

Substrate degradability for grass species estimated using the SNB method was generally lower than those estimated by NDF-digestion. This may be explained by the greater amount of microbial matter extracted from undegraded residue in the latter method (Blümmel and Becker, 1997). However, the degradation data estimate using the SNB method was similar to that determined by the in sacco method. Thus, in addition to the

measurement of volume of gas produced from the fermented substrate, the in vitro method may also be used in rapid estimation of DM degraded in sacco for large number of plants.

### 4.2. Addition of PEG and its effect on substrate degradability and gas production

PEG was chosen as a tannin-binding agent because PEG complexes with tannin more efficiently than PVP (Khazaal et al., 1993; Makkar et al., 1995a,b). In addition, due to its high solubility, virtually all PEG was washed out from the nylon bag and thus did not interfere with the residue weight. The use of the SNB method to estimate substrate degradability after 24 h incubation in vitro is important because the inhibitory effect of tannins on microbial fermentation and the degradability of substrate may be investigated simultaneously. For example, the SNB method developed allowed us to demonstrate changes in the substrate degradability resulting from addition of catechin, and catechin plus PEG.

Higher PF value (3.77) than that in control (3.37), though not significant, was noted when catechin was also present during the fermentation of straw (Table 3). The PF value thus highlight the disproportionate inhibitory effects of catechin on the degradation of DM (22.2%) compared to gas production (29.8%), which was again observed in studies using browse plants when PEG was also added into the incubation. Inclusion of PEG had no significant effect on wheat straw degradation (Table 3), but its presence increased the degradation of browse plants (Table 4). Regression analysis between browse and leguminous plants DM degradability using in vitro data was better with those determined by in sacco method when PEG was added to the incubation medium ($y = 0.71x + 14.9$; $r^2 = 0.92$) compared to data without PEG ($y = 0.59x + 15.0$; $r^2 = 0.72$). Better correlation in the former may be explained by the fact that most negative effects, which are intrinsic to browse plants tannins, were removed by PEG. It is interesting to note that the increase in degradability of browse plants, ranging from +1.7 to +34.4%, tended to be smaller than the changes in gas production, which ranged from +1.9 to 140.5%. This highlights the potential effect that addition of PEG to diets containing browse plants may enhance degradability of both the soluble and insoluble fractions.

### 4.3. PF values of substrates

End products of fermentation include SCFA, fermentative gases and microbial cells. The more substrate that is degraded by the rumen microbes, the greater energy that is made available as SCFA (measured partly as gas production) or to be used for the synthesis of microbial cells. Because the gas produced is inversely related to microbial yield (Blümmel et al., 1997a) the PF value reflects variations in microbial biomass yield. In the present study, the addition of PEG generally resulted in a much higher increase in gas production compared with substrate degradability, and thus a reduction of PF values ($PF_{+PEG}$). Although it is desirable to have an increase in substrate degradability when the effect of tannin is reduced, a simultaneous much larger increase in gas production would simply result lower partition of nutrients to microbial yield (Makkar et al., 1998). Increased gas production in the presence of PEG (Table 4) also provides further evidence that the presence of tannins in browse plants protect feed particles from extensive microbial degradation in the rumen, thus affecting the partition of nutrients which tend to enhance

protein microbial synthesis as opposed to SCFA production (Blümmel et al., 1997a). In addition, binding of tannin to feed protein increased protein availability post-ruminally (Waghorn et al., 1987) though this assumes that the tannin complexed with protein is reversible in the small intestine.

The theoretical range for PF values for tannin-free plants was suggested by Blümmel et al. (1997b) to be between 2.75 and 4.41 mg truly degraded substrate/ml gas. PF values greater than 4.41 are not theoretically possible (Makkar et al., 1998) and if this occurred, would simply indicate (i) significant loss of detached tannins from fermented substrate, which do not contribute to gas production, (ii) non-utilisation of soluble fraction due to tannins, or combination of (i) and (ii). In the present study, calculated PF values for browse plants were much higher and ranged from 5.05 to 11.05. This could be attributed to combination of factors (i), (ii) and a third factor, i.e. feed particles, which escaped from the nylon bag during washing and did not contribute to gas, either due to inhibitory tannin effects on substrate fermentation or particles which are insoluble and undegradable. These factors stress the importance of being cautious when looking at absolute PF values. The values, however, may be used in relative comparisons and can be particularly useful in the comparison of PF values within a tannin-containing plant (Table 4). It is interesting to note that the increase in gas production in the presence of PEG was not always supported by a proportional increase in substrate degradability. This suggests that there may be differences in the way inhibitory tannins from different browse plants affect the activity of rumen microbes. Thus, the measurement of both gas production and substrate degraded in the presence and absence of PEG, and the changes in calculated PF would provide useful insight into the degree of inhibitory effects of tannins on substrate fermentation by rumen microbes. Further studies using greater variety and number as well as stage of growth of browse plants are necessary to establish a more robust relationship between browse plants intrinsic properties (chemical composition, voluntary dry matter intake, maximum inclusion in the diet), and the PF and $PF_{+PEG}$ values.

## 5. Conclusions

In addition to the measurement of gas produced using the gas production in vitro method, the new validated method also enables the estimation of in vitro degradability. This method of measuring the differential in PF values with and without PEG provides a means of assessing to what degree the tannin content of a legume, or browse plant, is affecting its rumen fermentation. The method might be useful in preliminary evaluation of two different types of plant, one of which contain tannins, prior to considering the suitability of feeding these plants as a mixed ration.

## Acknowledgements

Financial supports by The British Council, Kuala Lumpur (CICHE 248) and The University of Malaya (UM) for the Rowett Research Institute-UM academic link is highly appreciated.

## References

AOAC, 1990. Official Methods of Analysis, 15th Edition. Association Official Analytical Chemists, Arlington, VA.

Blümmel, M., Becker, K., 1997. The degradability characteristics of fifty-four roughages and roughages neutral-detergent fibres as described by in vitro gas production and their relationship to voluntary feed intake. Br. J. Nutr. 77 (5), 757–768.

Blümmel, M., Ørskov, E.R., 1993. Comparison of in vitro gas production and nylon bag degradability of roughages in prediction of feed intake in cattle. Anim. Feed Sci. Technol. 40, 109–119.

Blümmel, M., Steingass, H., Becker, K., 1994. The partitioning of in vitro fermentation products and its bearing for the prediction of voluntary feed intake. Proc. Soc. Nutr. Physiol. 3, 123.

Blümmel, M., Makkar, H.P.S., Becker, K., 1997a. In vitro gas production: a technique revisited. J. Anim. Physiol Anim. Nutr. 77, 24–34.

Blümmel, M., Steingass, H., Becker, K., 1997b. The relationship between in vitro gas production, in vitro microbial biomass and N-15 incorporation and its implications for the prediction of voluntary feed intake of roughages. Br. J. Nutr. 77, 911–921.

Hungate, R., 1966. Rumen and its Microbes. Academic Press, London.

Jones, G.A., McAllister, T.A., Muir, A.D., Cheng, K.J., 1994. Effects of sainfoin (*Onobrychis viciifolia* Scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. Appl. Environ. Microbiol. 60, 1374–1378.

Julkunen-Tiito, R., 1985. Phenolics constituents in the leaves of Northern willows: methods for the analysis of certain phenolics. J. Agric. Food Chem. 33, 213–217.

Khazaal, K., Dentinho, M.T., Ribeiro, J.M., Ørskov, E.R., 1993. A comparison of gas production during incubation with rumen contents in vitro and nylon bag degradability as predictors of the apparent digestibility in vivo and the voluntary intake of hays. Anim. Prod. 57, 105–112.

Khazaal, K., Boza, J., Ørskov, E.R., 1994. Assessment of phenolic-related antinutritive effects in Mediterranean browse — a comparison between the use of the in vitro gas production technique with or without insoluble polyvinylpolypyrrolidone or nylon bag. Anim. Feed Sci. Technol. 49, 133–149.

Krishnamoorthy, U., Soller, H., Steingass, H.H., Menke, K.H., 1991. A comparative study on rumen fermentation of energy supplements in vitro. J. Anim. Physiol. Anim. Nutr. 65, 28–35.

Leng, R.A., 1993. Quantitative ruminant nutrition — a green science. Aust. J. Agric. Res. 44, 363–380.

Makkar, H.P.S., Blümmel, M., Becker, K., 1995a. Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins, and their implication in gas production and true digestibility in in vitro techniques. Br. J. Nutr. 73, 897–913.

Makkar, H.P.S., Sen, S., Blümmel, M., Becker, K., 1995b. Effects of fraction containing saponin from *Yucca schidigera*, *Qullaja saponaria*, and *Acacia auriculoformis* on rumen fermentation. J. Agric. Food Chem. 46, 4324–4328.

Makkar, H.P.S., Blümmel, M., Becker, K., 1998. Application of an in vitro gas method to understand the effects of natural plant products on availability and partitioning of nutrients. In: in vitro Techniques for Measuring Nutrient Supply to Ruminants. Br. Soc. of Anim. Sci., Occasional Publication no. 22, pp. 147–150.

Menke, K., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W., 1979. The estimation of digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. J. Agric. Sci. Cambridge 3, 217–222.

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. Cambridge 92, 499–503.

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

Robertson, J.B., Van Soest, P.J., 1981. The detergent system of analysis. In: James, W.P.T., Theander, O. (Eds.), The Analysis of Dietary Fibre in Food. Marcel Dekker, New York, pp. 123–158.

Salawu, M.B., Acamovic, T., Stewart, L.S., Hovell, F.D., 1997. Quebracho tannins with or without Browse Plus (a commercial preparation of polyethylene glycol) in sheep diets: effect on digestibility of nutrients in vivo and degradation of grass hay in sacco and in vitro. Anim. Feed Sci. Technol. 73, 99.

Steele, R.G.D., Torrie, J.H., 1980. Principles and Procedure of Statistical Analysis. McGraw Hill, New York.
Van Soest, P.J., Robinson, J.B., 1985. A Laboratory Manual for Animal Science. Cornell University, Ithaca, NY.
Waghorn, G.C., Ulyatt, M.J., John, A., Fisher, M.T., 1987. The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on Lotus corniculatus. Br. J. Nutr. 57, 115–126.