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The relationship between *in vitro* gas production, *in vitro* microbial biomass yield and ^{15}N incorporation and its implications for the prediction of voluntary feed intake of roughages

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The relationship between *in vitro* gas production, concomitant *in vitro* apparent and true DM degradability has been examined in forty-two roughages. The partitioning of truly-degraded substrate between gas volume and microbial biomass yield and ^{15}N incorporation into cells was also investigated. The relevance of this partitioning for the regulation of DM intake (DMI) was examined for fifty-four roughages. The results can be summarized as follows. *In vitro* gas production and *in vitro* apparent and true degradability are highly correlated ($P < 0.0001$), r being 0.96 and 0.95 respectively. There is an inverse relationship between *in vitro* gas production and microbial biomass yield (r −0.67, ($P < 0.0001$) and also ^{15}N enrichment ($P < 0.001$) when the variables were related to a given unit of substrate truly degraded. Selecting roughages by *in vitro* gas production may well be a selection against maximum microbial yield and a combination of *in vitro* gas volume measurements with a complementary determination of the substrate truly degraded is proposed, to calculate a partitioning factor (PF) reflecting the variation of short-chain fatty acid production per unit substrate degraded. PF is calculated as the ratio, substrate truly degraded : gas produced by it. PF was highly significant ($P < 0.0001$) in DMI prediction when included in stepwise multiple correlations together with *in vitro* gas volume variables reflecting the extent and rate of gas production; 11 % of the variation in DMI was accounted for by the PF. The total model, including extent and rate of gas production and the PF, accounted for 84 % of the variation in DMI. Roughages producing proportionally less gas per unit substrate truly degraded had higher feed intakes.

Gas production: Microbial activity: ^{15}N incorporation: *In vitro* degradability: Voluntary feed intake

The association between rumen fermentation and gas production has long been known (Tappeiner, 1884 as cited by Marston, 1948). Quin (1943) examined the fermentation of glucose, lucerne (*Medicago sativa*) hay and various types of straw by connecting a manometer device for the recording of gas production directly to the rumen cannulas of Merino sheep. Finding this technique too difficult to execute and of poor reproducibility, he switched to the measurement of *in vitro* gas production. The concept was taken up by McBee (1953) who refined the technique (he used a Warburg respirator) to investigate the fermentation of cellulose and hemicellulose. Further variations of the technique have been developed over the years, examining the different aspects of rumen fermentation (Hungate *et al.* 1955; Czerkawski & Breckenridge, 1970; Trei *et al.* 1970; O'Hara & Okhi, 1973).

More recently, the rising interest in the efficient utilization of roughages, particularly in developing countries, has caused an upsurge in interest in *in vitro* gas production tests, since in these tests the kinetics of fermentation can be estimated using one sample. The kinetics of fermentation are thought to be linked to voluntary feed intake, widely considered the severest constraint in roughage utilization.

Much of the recent work on *in vitro* gas production systems has been devoted to the automation of gas volume recordings (Beuvink *et al.* 1992; Pell & Schoefield, 1993) and to the derivation of mathematical relationships which describe the kinetics of gas production (Krishnamoorthy *et al.* 1991; Beuvink & Kogut, 1993; France *et al.* 1993). Less attention was given to what gas production reflects and how gas production is related to concomitant *in vitro* true and apparent degradabilities.

Blümmel & Ørskov (1993) described *in vitro* gas volumes for ten roughages by the amount and proportion of acetate, propionate and butyrate present in the system; gas volumes were produced according to the stoichiometry of Wolin (1960). In the same work Blümmel & Ørskov (1993) pointed out that the *in vitro* gas production test based on bicarbonate-buffer basically-titrated short-chain fatty acid (SCFA) production measured fermentation-derived CO_2 and CH_4 and CO_2 produced upon buffering the generated SCFA. SCFA represent one important fermentation product, the other being microbial cells. The partitioning of fermented matter between SCFA and microbial cells is not constant (Stouthamer, 1973; Hespell & Bryant, 1979; Harrison & McAllan, 1980; Tempest & Neijssel, 1984).

The present work was undertaken to examine the hypothesis that an inverse relationship exists between gas or SCFA production and microbial biomass yield (Kristensen & Weisbjerg, 1991; Leng, 1993; Blümmel *et al.* 1994).

MATERIAL AND METHODS

Preparation of inoculum and in vitro *device used*

The procedures followed were similar to those described by Blümmel & Becker (1997) in the previous paper for the incubation of 500 mg substrate in the modified Hohenheim gas production test.

Analysis of in vitro *apparent degradability*

The incubation was terminated at a chosen time, in this case after 24 h of incubation, the gas volume was recorded and the whole contents of the syringe were transferred into pre-tared 70 ml capped centrifuge tubes (Polycarbonate; Beckman, Munich, Germany). The residue was centrifuged in an ultracentrifuge at 20 000 *g* for 30 min at 4°. The supernatant fraction was carefully removed with a pipette, frozen and stored for NH_3 analysis (see p. 913). The incubation syringes were washed three times with a total of about 60 ml NaCl (4 g/l) solution. The washing solution was injected through the spike of the syringes using a dispenser (syringe and plunger were not separated after terminating the incubation). The washing solution was emptied each time from the syringe into the centrifuge tube containing the pellet of the first centrifugation. The tubes were shaken gently, the pellet loosened and redispersed in the tubes. The tubes were again centrifuged at 20 000 *g* for 30 min at 4°, opened and the supernatant fraction discarded. The residual pellet was pre-frozen and lyophilized in the tubes overnight. Residual moisture was removed by drying the tubes at 105° for 3 h in a forced-draft drying oven. The tubes were weighed and the

apparent undegradability was calculated as the mass of the full centrifuge tubes minus that of empty tubes and corrected for the pellet mass of the blank incubations.

Analysis of in vitro *true degradability*

In vitro true degradabilities were determined by refluxing the pellet contained in the centrifuge tubes to separate the microbial mass from the undegraded substrate. To achieve this, the pellet was transferred from the tubes into 600 ml spoutless beakers and the centrifuge tubes were thoroughly washed with about 70 ml neutral-detergent solution (NDS; Van Soest & Robertson, 1985) which was added to the beakers. The pellet was refluxed for 1 h and the residue was recovered in pre-tared filter crucibles (determination of the true degradability, by the method of Van Soest & Robertson, 1985). True degradability was calculated as the weight of substrate incubated minus the weight of the residue after NDS treatment.

Measurements of ^{15}N incorporation into microbial cells

An analogous approach to the one described previously was followed. The N in the NH_4HCO_3 buffer, however, consisted of 96·5% ^{15}N-enriched N (Berlin Chemie, Adlershof, Germany). The pellet was prepared as described previously and was powdered in a vibratory disc mill (Siebtechnik, Germany), and when necessary rehomogenized with a spatula. An amount of sample approximately equivalent to about 80–120 μg N (about 0·6–3·2 mg dry residue) was weighed into tin capsules (Europa Scientific Ltd, Crewe, Cheshire) using a micro balance (Mettler, Germany). Total N as well as ^{15}N enrichment was measured using a mass spectrometer (Tracermass, Stable Isotope Analyser; Europa Scientific Ltd) combined with an element analyser (Biological Sample Converter, Robopreb-CN; Europa Scientific Ltd). A standard (^{15}N-labelled NH_4Cl; Berlin Chemie) of known ^{15}N enrichment (13·1%) was included in the measurements. Enrichment of ^{15}N was calculated on the basis of the total N content of the residual pellet (apparently-undegraded pellet) according to

$$\text{mg}^{15}\text{N} = \text{mg pellet} \times (\%\,\text{N}/100) \times (\%^{15}\text{N}/100),$$

where mg pellet is the weight of the undegraded pellet, %N is percentage N in the undegraded pellet and % ^{15}N is ^{15}N as a percentage of total N.

Total N and ^{15}N in the blank incubations were subtracted to obtain net ^{15}N incorporations.

Analysis of cell-wall-bound (neutral-detergent fibre; NDF) nitrogen and ammonia-nitrogen

For NDF N analysis, the residue was transferred directly from the filter crucibles into Kjeldahl digestion tubes; the exact sample weight was determined by hot weighing the crucibles before and after removal of the residue (Van Soest & Robertson, 1985).

NH_3-N was determined in the supernatant fraction by steam distillation; 2 ml 1 M-NaOH was added to 5 ml of the supernatant fraction diluted with 30 ml water and the solution was directly distilled and NH_3 evolved was collected into boric acid (30 g/l). The distillate was titrated with 0·05 M-H_2SO_4.

Substrates used for the in vitro *incubations*

The roughages used have been described in our previous paper (Blümmel & Becker, 1997). Total fibre (NDF) content ranged from 648 to 875 g/kg (average 772 (SD 6·3) g/kg) and the N content ranged from 2 to 15 g/kg (average 7·3 (SD 0·48) g/kg).

Statistical analysis

The computer programme SAS/STAT (Statistical Analysis Systems, 1988) was used for the multiple-regression procedures (see Blümmel & Becker, 1997), and also for the comparison of ^{15}N supply and ^{15}N pellet enrichment by paired t test. Linear regressions were calculated using GraphPad Inplot (GraphPad Software, 1990).

RESULTS

Gas volume and in vitro *true and apparent degradability*

The air-dry sample (500 mg; 460 mg DM) was incubated and 53·3–223·3 mg (average 134·0 mg) were apparently degraded. The volume of gas produced ranged from 26·1 to 86·8 ml (average 58·6 ml). The relationship between gas volume and substrate degradability *in vitro* is presented in Fig. 1. A highly significant correlation (r 0·95, $P < 0·0001$) was found between gas produced and substrate apparently degraded. The intercept of the regression equation (5·0) was not significantly ($P > 0·05$) different from zero and the regression coefficient indicated that 1 mg degraded substrate produced 0·4 ml gas (Fig. 1).

Gas volumes and substrate true degradabilities (Fig. 1) were also highly correlated (r 0·96, $P < 0·0001$). The amount of substrate degraded ranged from 128·0 to 336·9 mg (average 237·6 mg). The intercept (−11·3) of the regression equation was significantly

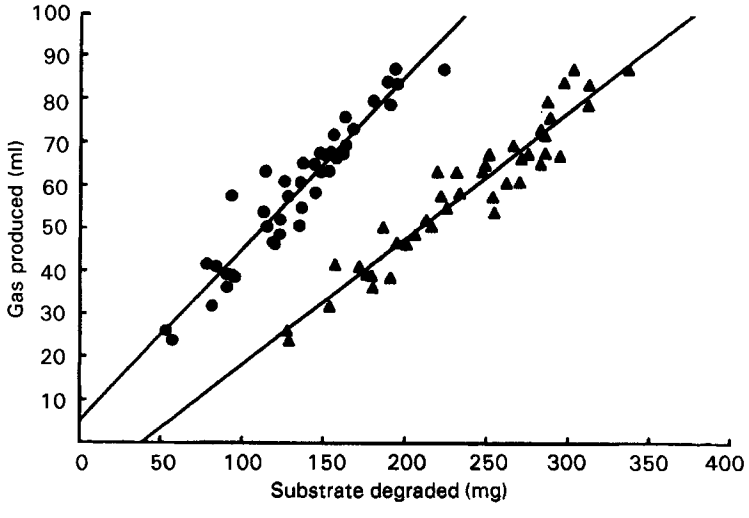

Fig. 1. Relationship between DM apparently (●) and truly (▲) degraded (mg) and volume (ml) of gas produced after the incubation of 500 mg substrate for 24 h. For details of substrates and procedures, see pp. 912–913. DM apparently degraded: $y = 5·0 + 0·40x$, r 0·95, DM truly degraded: $y = -11·3 + 0·29x$, r 0·96.

($P < 0.01$) different from zero and 1 mg truly-degraded substrate produced 0·29 ml gas (Fig. 1).

Gas volume, microbial yield and the partitioning of fermentation products

The mass of material solubilized from the pellet obtained following centrifugation of syringe contents after 24 h fermentation by NDS treatment (amount of substrate truly degraded – amount apparently degraded) was calculated and used as an estimate of microbial biomass (average 103·6 mg). An inverse relationship existed between amount of microbial biomass and the volume of gas produced per 100 mg substrate truly degraded (Fig. 2).

There was a significant (r −0·67, $P < 0.0001$) negative correlation between gas production and amount of biomass from a given unit amount of substrate truly degraded. Biomass levels ranged from 32·7 to 55·7 mg/100 mg truly-degraded matter, and gas volumes varied from 20·1 to 37·7 ml. Gas volume per 100 mg was highest with cellulose and biomass was lowest with this substrate. Omitting cellulose from the statistical analysis in Fig. 2 did not significantly change the relationship (r −0·64, $P < 0.0001$) reported in Fig. 2.

^{15}N enrichment

Seventeen of the forty-two substrates (without cellulose) were used to compare the gravimetric estimation of biomass by centrifugation and NDS treatment with ^{15}N enrichment. For this study, the unlabelled NH_4HCO_3-N in the standard medium was replaced with ^{15}N-enriched NH_4HCO_3 (96·5 atom % ^{15}N). The N supply from the various

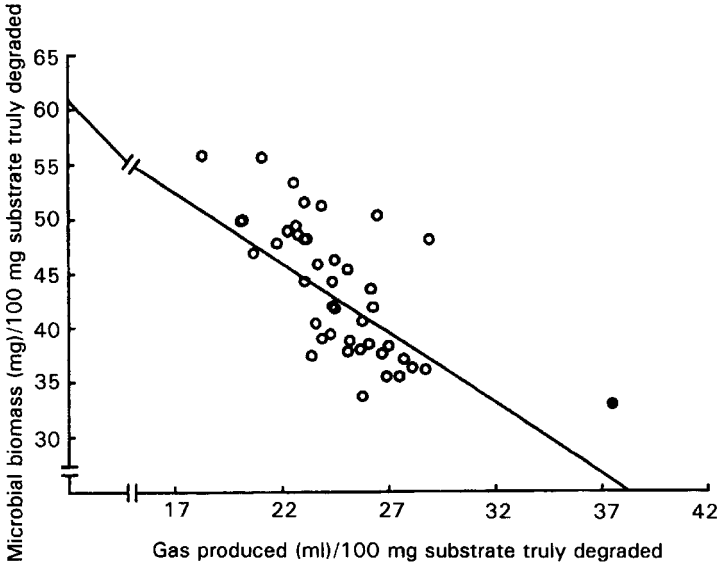

Fig. 2. Relationship between volume (ml) of gas produced per 100 mg substrate truly degraded and microbial biomass generated (mg). (○), Roughages; (●), cellulose. For details of substrates and procedures see pp. 912–914.

Table 1. *Mean values for nitrogen (mg) from various sources during the* in vitro *incubation of 500 mg substrate in 40 ml of a ^{15}N-enriched suspension of rumen fluid**

NH_3-N (mg)			Substrate N (mg)	^{15}N (% total N)
Buffer N	Rumen-fluid N	Lysis N		
7·10 (6·85 ^{15}N)	1·00	2·00	3·67†	49·7

* For details of substrates and procedures, see pp. 912–913.
† Substrate N minus undegraded neutral-detergent-fibre N.

sources, i.e. medium N, rumen fluid N and lysis N (N increase in 24 h in the blank incubations) is presented in Table 1.

In the pellet obtained at 24 h, ^{15}N accounted for 37·7 (SD 3·1) % of the net N (total pellet N minus blank pellet N minus NDF N). These observations show that enriched N was incorporated into cells in a proportion significantly ($P < 0.0001$) smaller than that present in the system (49·7 %).

^{15}N yield in the pellet correlated well ($P < 0.0001$) with biomass yield, as determined by centrifugation and NDS treatment, suggesting good agreement between the measurements. The relationship can be expressed by the linear-regression equation:

$$\text{biomass yield (y)} = 24{\cdot}5 + 46{\cdot}3^{15}\text{N}, r\ 0{\cdot}93\ (\text{Sy} \times 6{\cdot}95),$$

where ^{15}N and biomass yield are expressed as mg.

^{15}N enrichment and gas production

Substrates with proportionally high ^{15}N incorporation per 100 mg substrate truly degraded produced less gas than substrates with lower ^{15}N yields. The inverse relationship (Fig. 3) was significant (r −0·77, $P < 0.001$).

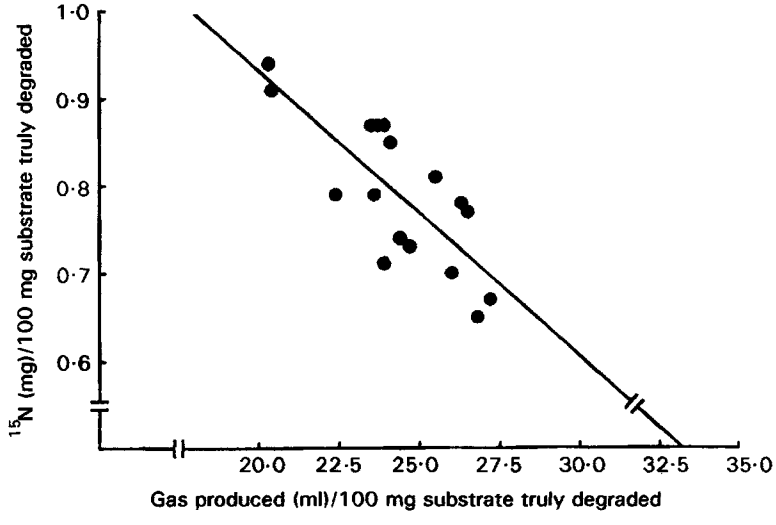

Fig. 3. Relationship between volume of gas produced per 100 mg of substrate truly degraded and ^{15}N incorporated into microbial biomass (mg). For details of substrates and procedures see pp. 912–914. $y = 1{\cdot}59 - 0{\cdot}033x$, r −0·77 (n 17).

DISCUSSION

Apparent degradability, gas volume and stoichiometry

Apparent degradability and gas volume were well (r 0·95, $P < 0·0001$) correlated (Fig. 1). The conversion of apparently-degraded substrate, as determined by high-speed centrifugation, into gas is in agreement with stoichiometrical considerations. Blümmel & Ørskov (1993) showed with ten types of straw examined at 24 and 48 h, substrates which are also part of this study, that gas volumes in the *in vitro* fermentation of the Hohenheim gas test could be explained by the amount and proportions of acetate, propionate and butyrate present in the system. From the SCFA the production of CO_2 and CH_4 could be estimated by the stoichiometry of Wolin (1960), which has been validated recently (Blümmel *et al.* 1993), where measured SCFA and measured gas volume, and calculated and measured gas volume were well correlated (r 0·99 and 0·99, $P < 0·0001$ respectively). The average composition of 1 mmol SCFA in the previously described fermentation was about 0·7 acetate, 0·21 propionate and 0·09 butyrate and led to the formation of 0·538 mmol CO_2 and 0·348 mmol CH_4 (Blümmel & Ørskov, 1993). A summation of the molecular weight of the acids, CO_2, CH_4 and water (produced by the formation of CH_4 according to $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$) in the described proportions reveals the need for at least 106·9 mg C, O and H. One mmol SCFA will lead to 48·2 ml gas, where 13·8 ml CO_2 and 8·8 ml CH_4 are derived directly from the fermentation and 25·6 ml CO_2 are derived from the buffering of SCFA (Blümmel & Ørskov, 1993).

Inserting the stoichiometrically-calculated factor of 106·9 mg into the equation ($y = 5·0 + 0·4x$) in Fig. 1, using the regression gas volume *v.* apparently-degraded substrate, would lead to the production of 47·8 ml gas. Alternatively, inserting the stoichiometrically-calculated gas volume of 48·2 ml as the y value into the same equation would suggest 108·0 mg to be apparently degraded. These results show very good agreement between stoichiometrical considerations and experimental evidence and support the assumptions that the apparently-degraded substrate was used for the production of SCFA and the fermentation-derived CO_2, CH_4 and water were produced at the same time.

Centrifugation at 20 000 ***g*** is routinely used to separate microbial cells from suspensions of rumen fluid (Crawford *et al.* 1980; Dahlberg *et al.* 1988; Windschitl & Stern, 1988*a*, *b*; Bas *et al.* 1989). Feed particles and protozoa are already sedimented at 100–2000 ***g*** (Krawielitzki & Voigt, 1988; Krawielitzki *et al.* 1989). The pellet, therefore, should consist of microbial biomass and undegraded substrate. The good agreement between the stoichiometrically-calculated conversion of apparently-degraded substrate into gas volume and apparent substrate degradation *v.* that determined support that assumption. These findings suggest a high recovery of biomass by the centrifugation process.

Gas volume, true degradability and biomass yield

Refluxing the apparently-undegraded residues with NDS is considered to be effective in extracting the microbial biomass from the residue, leaving the undegraded feed (Mason, 1979; Van Soest, 1994), thus providing an estimate of biomass yield. There is a close relationship (r 0·96, $P < 0·0001$) between gas volumes and true degradabilities (Fig. 1). A substantial amount (40·0 mg) of substrate, however, appears to have been degraded without any gas being produced. This amount of substrate has been used for purposes other than SCFA production.

There was a significant (r −0·67, $P < 0·0001$) negative relationship between gas produced and microbial biomass generated when both were related to 100 mg truly-

degraded substrate (Fig. 2). There are two potential errors in the quantification of microbial biomass by NDS treatment; more than just the microbes, or not all the microbes, may be removed. However, NDS solubles from straw are considered to be completely fermented at earlier stages of incubation, and analysis of the NDS-soluble fraction did not suggest significant microbial contamination (Mason, 1979; Van Soest, 1994). After NDS treatment of the Solka-Flok cellulose residue incubated for 24 h (included in Fig. 2) 0·32 g N/kg remained. Refluxing with distilled water instead of NDS removed microbial mass less efficiently, since the residue contained 4·5 g N/kg. These findings are in agreement with results reported for water treatments of *in sacco* residues, where ^{35}S (Kennedy *et al.* 1984) or ^{15}N determinations (Varvikko & Lindberg, 1985) revealed substantial residual microbial contamination.

NDS treatment removed an average of 103·6 mg from the pellets. The regression equation (Fig. 1) indicates that about 40 mg substrate were truly degraded without leading to gas production, which is equivalent to about 40 % of the average mass solubilized by NDS. Clearly, relative to 1 ml gas, more substrate is truly degraded than apparently degraded, since the former measurements also account for microbial biomass production as well as SCFA and fermentation derived CO_2, CH_4 and water; microbial biomass production would explain the different ($P < 0·001$) slopes and intercepts of the two regression equations presented in Fig 1. Considering the nature of the substrates, which were mostly types of Gramminaceous straw, it seems unlikely that NDS-solubilized but undegraded substrate would contribute to these differences to any significant extent. For this type of material, NDS treatment is a widely-used and accepted method for the determination of true digestibilities, i.e. separating microbial biomass from the undegraded substrate (Van Soest & Robertson, 1985; Van Soest, 1994).

^{15}N enrichment and gas production

While refluxing with NDS was shown to remove microbes efficiently from the undegraded feed, it cannot be excluded that NDS treatment occasionally removes more than just microbes, which would then, however, contribute to the difference between apparent and true substrate degradability, as presented in Fig. 1. Thus, isotope studies with ^{15}N were conducted to validate quantitatively the previously mentioned relationship between gas production and biomass yield. The inverse relationship found between these variables does not seem to be due to a systematic error in the estimation of microbial biomass by centrifugation and NDS treatment. Incorporation of ^{15}N into cells was also negatively (r $-0·77$, $P < 0·001$) related to gas production when both were expressed per 100 mg substrate truly degraded (Fig. 3). The biomass, as quantitatively estimated by centrifugation and NDS treatment, was significantly correlated with the ^{15}N content (r 0·93, $P < 0·001$) of the pellet, suggesting that estimation of biomass yield as the difference in the weight of the pellet obtained on centrifugation and that of the residue following NDS treatment was acceptable. These results support the assumption that the material solubilized from the pellet by NDS was of microbial origin.

Suggested modification in the use of in vitro *gas production*

In vitro gas production accurately reflects substrate fermentation to SCFA (Blümmel & Ørskov, 1993; Blümmel *et al.* 1993), which was shown for ten types of straw (these were also included in the seventeen feeds used in the present ^{15}N study); the other important fermentation products are microbial cells. The *in vitro* partitioning of fermented matter

Table 2. *Stepwise multiple correlations* (R^2) *between* A, B, c *and partitioning factor (PF) and DM intake (DMI) of fifty-four roughages*

Parameter	*Y* variate	R^2	Prob > F
$A+B+c+$PF*	DMI (g/kg live wt per d)	0·838	0·0001
B		0·462†	0·0001
A		0·222†	0·0001
PF		0·111†	0·0001
c		0·043†	0·0007

A, B, c Constants in the *in vitro* gas-production model $y=A+B(1-e^{-ct})$ when applied to 200 mg substrate for 96 h.
* Calculated from the incubation of 500 mg substrate for 24 h as the ratio, substrate truly degraded : gas produced.
† Partial R^2.

between SCFA and microbial cells is obviously not constant, which to some extent imposes an inverse relationship on both variables. Use of the *in vitro* gas production test might select against maximum microbial yield by favouring substrates with proportionally-high SCFA yield. This intrinsic disadvantage in *in vitro* gas production can be overcome by combining gas measurements with determination of the undegraded residue. The determination of the truly-undegraded substrate reveals the amount of substrate which was totally available to fermentation and the gas volume indicates the proportion of this substrate used for the SCFA. A variation in the relationship reflects the variation in microbial yield per unit SCFA produced. This variation can be expressed as a partitioning factor (PF), which is calculated as the ratio substrate truly degraded : gas volume produced (Blümmel *et al.* 1994).

Further information relating to the substrate, i.e. proportion of substrate fermented which contributes to the biomass production, can be obtained from PF.

This can be demonstrated by the recalculation of data from Blümmel & Becker (1997) who used a combination of *in vitro* gas production and residue (NDS treated) measurements at 24 h incubation to predict the DM intake (DMI) of fifty-four roughages. For these fifty-four roughages we calculated the ratio, substrate truly degraded : gas volume produced by it, i.e. PF, at 24 h incubation.

The factor so calculated, i.e. PF, was highly significant in the prediction of voluntary feed intake (Table 2) when applied to the intake prediction of fifty-four roughages. PF accounted for 11·1 % of the variation in DMI when combined with kinetic parameters of *in vitro* gas production *A, B* and *c* (see also Blümmel & Becker, 1997) and increased the percentage variation in DMI which was accounted for by from 75 % (Blümmel & Becker, 1997) to 84 % (Table 2). Roughages with a high PF, i.e. low gas production per unit truly degraded substrate had higher intakes.

PF was not significantly related to *in vitro* gas production or substrate degradability but was positively influenced by the N content of the roughages; however, this characteristic accounted for only about 18 % of the variation in PF.

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REFERENCES

Bas, F. J., Stern, M. D. & Merchen, N. R. (1989). Influence of protein supplementation of alkaline hydrogen peroxide-treated wheat straw on ruminal microbial fermentation. *Journal of Dairy Science* **72**, 1217–1227.

Beuvink, J. M. W. & Kogut, J. (1993). Modelling gas production kinetics of grass silages incubated with buffered ruminal fluid. *Journal of Animal Science* **71**, 1041–1046.

Beuvink, J. M. W., Spoelstra, S. F. & Hogendorp, R. J. (1992). An automated method for measuring time-course of gas production of feedstuffs incubated with buffered rumen fluid. *Netherlands Journal of Animal Science* **40**, 401–407.

Blümmel, M. & Becker, K. (1997). The degradability characteristics of 54 roughages and roughage NDF as described by *in vitro* gas production and their relationship to voluntary feed intake. *British Journal of Nutrition* **77**, 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. *Animal Feed Science and Technology* **40**, 109–119.

Blümmel, M., Steingaß, H. & Becker, K. (1994). The partitioning of *in vitro* fermentation products and its bearing for voluntary feed intake. *Proceedings of the Society of Nutrition Physiology* **3**, 123 Abstr.

Blümmel, M. Steingass, H., Becker, K. & Koppenhagen, M. (1993). Produktion von SCFA, CO_2, CH_4 und Mikroben bei der *in vitro* Fermentation im Hohenheimer Futterwerttest (Production of SCFA, CO_2, CH_4 and microbial cells *in vitro*). *Proceedings of the Society of Nutrition Physiology* **1**, 9 Abstr.

Crawford, R. J., Hoover, W. H. & Junkins, L. L. (1980). Effects of solids and liquids flows on fermentation in continuous culture. 2. Nitrogen partition and efficiencies of microbial synthesis. *Journal of Animal Science* **51**, 986–993.

Czerkawski, J. W. & Breckenridge, G. (1970). Small apparatus for studying rumen fermentation *in vitro*. *Laboratory Practice* **19**, 717–728.

Dahlberg, E. M., Stern, M. D. & Ehle, F. R. (1988). Effects of forage source on ruminal microbial nitrogen metabolism and carbohydrate digestion in continuous culture. *Journal of Animal Science* **66**, 2071–2083.

France, M., Dhanoa, M. S., Theodorou, M. K., Lister, S. J., Davies, D. R. & Isacs, D. (1993). A model to interpret gas accumulation profiles associated with *in vitro* degradation of ruminant feeds. *Journal of Theoretical Biology* **163**, 99–111.

GraphPad Software (1990). *GraphPad InPlot Version* 3.1. San Diego, CA: GraphPad Software.

Harrison, D. G. & McAllan, A. B. (1980). Factors affecting microbial growth yield in the reticulum rumen. In *Digestive Physiology and Metabolism in Ruminants*, pp. 205–225 [Y. Ruckebusch and P. Thivend, editors]. Lancaster: MTP Press.

Hespell, R. B. & Bryant, M. P. (1979). Efficiencies of rumen microbial growth: influence of some theoretical and experimental factors on ^{Y}ATP. *Journal of Animal Science* **49**, 1640–1659.

Hungate, R. E., Fletcher, D. W., Dougherty, R. W. & Barrentine, B. F. (1955). Microbial activity in the bovine rumen; its measurement and relation to bloat. *Applied Microbiology* **3**, 161–169.

Kennedy, P. M., Hazelwood, G. P. & Milligan, L. P. (1984). A comparison of methods for the estimation of the proportion of microbial nitrogen contamination in nylon bags incubated in the rumen of sheep. *British Journal of Nutrition* **52**, 403–417.

Krawielitzki, R. & Voigt, J. (1988). Der Einfluß unterschiedlicher Zentrifugationsbedingungen bei der Isolierung gemischter Pansenbakterien auf den Gehalt an Stickstoff und Diaminopimelinsäure (The influence of different conditions of centrifugation for isolation of mixed rumen bacteria on their content of nitrogen and diaminopimelic acid). *Archive of Animal Nutrition* **38**, 1–11.

Krawielitzki, R., Voigt, J. & Platkowski, B. (1989). Der Einfluß unterschiedlicher Zentrifugationsbedingungen bei der Isolierung gemischter Pansenbakterien auf deren Gehalt an Stickstoff und Diaminopimelinsäure: Verwendung von Duodenalchymus als Ausgangsmaterial (The influence of different conditions of centrifugation for isolation of mixed rumen bacteria on their content of nitrogen and diaminopimelic acid: use of duodenal content as source for isolation of rumen bacteria). *Archive of Animal Nutrition* **39**, 813–821.

Krishnamoorthy, U., Soller, H., Steingass, H. & Menke, K. H. (1991). A comparison study on rumen fermentation of energy supplements *in vitro*. *Journal of Animal Physiology and Animal Nutrition* **65**, 28–35.

Kristensen, V. F. & Weisbjerg, M. R. (1991). New approach to feed evaluation for ruminants. *Norwegian Journal of Agricultural Sciences* Suppl. 5, 67–81.

Leng, R. A. (1993). Quantitative ruminant nutrition – a green science. *Australian Journal of Animal Research* **44**, 363–380.

McBee, R. H. (1953). Manometric method for the evaluation of microbial activity of rumen with application to utilization of cellulose and hemicelluloses. *Applied Microbiology* **1**, 106–110.

Marston, H. R. (1948). The fermentation of cellulose *in vitro* by organism from the rumen of sheep. *Biochemical Journal* **42**, 564–574.

Mason, V. C. (1979). The quantitative importance of bacterial residues in the non-dietary faecal nitrogen of sheep. *Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde* **41**, 131–137.

O'Hara, M. & Okhi, K. (1973). Studies of the mode of gas production in an artificial rumen and its application to the evaluation of feedstuffs. *Japanese Journal of Zootechnical Science* **44**, 432–439.

Pell, A. N. & Schoefield, P. (1993). Computerizing monitoring of gas production to measure forage digestion *in vitro*. *Journal of Dairy Science* **76**, 1063–1073.

Quin, J. I. (1943). Studies on the alimentary tract of merino sheep in South Africa, 7: fermentation in the forestomachs of sheep. *Onderspoor Journal of Veterinary Science and Animal Industry* **2**, 91–117.

Statistical Analysis System (1988). *SAS/STAT Version 6.1* Cary, NC: SAS Institute Inc.
Stouthamer, A. H. (1973). A theoretical study on the amount of ATP required for synthesis of microbial cell material. *Antonie van Leeuwenhoek* **39**, 545–565.
Tempest, D. W. & Neijssel, O. M. (1984). The status of ^{Y}ATP and maintenance energy as biologically interpretable phenomena. *Annual Review of Microbiology* **38**, 459–486.
Trei, J., Hale, W. H. & Theurer, B. (1970). Effect of grain processing on *in vitro* gas production. *Journal of Animal Science* **30**, 825–831.
Van Soest, P. J. (1994). *The Nutritional Ecology of the Ruminant.* 2nd ed. Ithaca, NY: Cornell University Press.
Van Soest, P. & Robertson J. B. (1985). *A Laboratory Manual for Animal Science 612.* Ithaca, NY: Cornell University Press.
Varvikko, T. & Lindberg, J. E. (1985). Estimation of microbial nitrogen in nylon bag residues by feed ^{15}N dilution. *British Journal of Nutrition* **54**, 473–481.
Windschitl, P. M. & Stern, M. D. (1988*a*). Effect of urea supplementation of diets containing lignosulfate treated soybean meal on bacterial fermentation of ruminal contents. *Journal of Animal Science* **66**, 2948–2958.
Windschitl, P. M. & Stern, M. D. (1988*b*). Influence of methionine derivates on effluent flow of methionine from continuous culture of ruminal bacteria. *Journal of Animal Science* **66**, 2937–2947.
Wolin, M. J. (1960). A theoretical rumen fermentation balance. *Journal of Dairy Science* **43**, 1452–1459.