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A Review of Some Alternative Techniques to the Determination of Nutrient Digestibility for Ruminant Animals

Sebastian Chakeredza[1/] *Udo ter Meulen*[1/] *and Therdchai Vearasilp*[2/]

Abstract : To estimate organic matter and feed nutrient digestibilities and therefore the energy contents of feeds for ration formulation purposes, *in vitro* digestibility studies have to be conducted. However, *in vivo* studies are laborious, expensive and time consuming and are not suitable for routine feed evaluation use. In this paper, we review alternative techniques to the assessment of feed nutrient digestibility for ruminant animals. Chemical constituents and *in vitro* techniques like the Tilley and Terry, nylon bag, gas production and cellulase methods are some of the most commonly used alternative methods. Use of chemical constituents for prediction purposes is imprecise. Good agreement of results has been found between the other four methods in most of the feeds evaluated. Because the cellulase method is non-invasive, simple, cheap, fast and precise, it is the preferred method for routine feed evaluation. Further studies should however be conducted to improve its precision across a wider rage of feeds.

Key words : *In vitro* methods, Tilley and Terry, Gas method, Nylon bag, Cellulase method

[1/] Institute of Animal Physiology and Nutrition, Georg August University, 37077 Gttingen, Germany.

[2/] Department of Animal Science, Faculty of Agriculture, Chiang Mai Univrsity, Chiang Mai 50200, Thailand

INTRODUCTION

For ration formulation purposes, knowledge of the feeding value of feed ingredients, which make up a ration, is imperative. To have such data, it is necessary to determine the digestibility of the individual nutrients. Almost all energy systems currently in use world-wide require the digestible organic constituent values of individual feed components. These values are then used to formulate a ration to meet a particulartive function.

In vivo digestibility measurements with animals are expensive, time consuming and require large quantities of feed. They are therefore not suitable for routine digestibility assessments.

Alternative procedures which have been used to estimate nutrient organic matter digestibilities include laboratory chemical methods, two stage *in vitro* technique (Tilley and Terry 1963), gas production (Menke *et al.*, 1979), *in sacco* degradability using cannulated animals (Ørskov and McDonald, 1979) and various enzymatic methods (De Boever *et al.*, 1988)

Due to concerns of animal welfare, methods that are non-invasive, rapid, inexpensive and precise are to be promoted. This review gives a critical examination of the alternative methods to *in vivo* digestibility for the prediction of nutrient digestibilities, which are currently in use. The chemical, the two-stage Tilley and Terry, gas production, *in sacco* (nylon bag) and cellulase methods are covered. Their advantages and disdvantages and scope for wider application are highlighted.

IN VIVO METHOD

The direct measurement of digestibility is a laborious method. Three or four animals, kept in metabolism cages are given the same weighed amounts of the same feed or feed mixture for four weeks. During the last 7-10 days of the trial, collections are made of faeces, which are weighed, sampled and analysed, like the feed for their nutrient content. Besids being laborious, this method requires large quantities of feeds, is expensive and time consuming.

Factors which need to be considered when conducting an *in vivo* digestibility study are; the species, breed, sex, liveweight and health status of the animals, diet offered the animals in terms of the energy, roughage : concentrate ratio, crude protein content and mineral requirements and the prevailing environmental conditions. A review of these factors has been published by the committee for standards on animal nutrient requirements, Society for Nutrition Physiology, Frankfurt, Germany (Ausschuβ für Ernährungsphysiologie, 1991).

It will be important to standardise the measurement of the *in vivo* digestibility before other alternative methods can fairly be evaluated especially if the *in vivo* results are going to be used as the baseline. Assuming that the *in vivo* digestibility as-

sessments are carried out under standard conditions, to circumvent the other limitations of the *in vivo* digestibility determination, laboratory or chemical methods and *in vitro* techniqes have been developed.

CHEMICAL ANALYTICAL METHODS

A lot of attention is still paid to the derivation of relationships between the chemical characteristics and the digestibility of feedstuffs. The Proximate scheme or Van Soest detergent systems of analysis are used for the prediction of the digestibility of the organic matter and estimate the energy contents (Van Soest 1982). Determinations of crude fibre, crude protein and cell wall constituents are normally carried out. Using these constituents as independent variables overlooks the fact that their determinations are not accurate. In addition, variations in conditions during plant growth or during processing may affect the relationship between the Proximate and Van Soest detergent constituents and digestibility. Most of the equations generated relating *in vivo* digestibility with chemical constituents are associated with a high residual standard deviation which makes their general application limited (De Boever *et al.*, 1996). In most cases, *in vitro* techniques are normally adopted to estimate *in vivo* digestibility values.

IN VITRO TECHNIQUES

Tilley and Terry method

The single or two-stage Tilley and Terry (1963) system is the commonly used *in vitro* digestion system. This method involves sample incubation with rumen fluid and a buffering media under anaerobic conditions in the dark at 38°C for 48 hours. This is to simulate rumen digestion. This is followed by a second 48 hour digestion period using pepsin and weak acid to remove undigested protein. Using 146 samples of grass, clover and lucerne of known *in vivo* digestibility (Y), the regression equation $Y = 0.99X - 1.01$ (S.E. ± 2.31) was established with X being the *in vitro* dry matter digestibility in per cent. Organic matter and individual nutrient digestibilities can also be determined. To translate the *in vivo* organic matter digestibilities (IVDOM) to metabolisable energy (ME), the following relationship of Barber *et al.* (1984) can be used : ME (MJ/Kg DM) = 0.0157 [IVDOM], where IVDOM is in g/kg DM.

This method uses simple apparatus. It has been found to be reproducible and many samples can be handled in a single experiment. Time of collection of rumen fluid and the diet fed to the animals have been found to have effects on the results obtained (Cone *et al.*, 1989).

Major disadvantages to the method are the number of steps and length of time required for the analysis. In addition, the necessity to maintain fistulated donor animals for microbial inoculum is a restriction to the wider application of the technique. Instead of assessing dry matter or organic matter disappearance, techniques, which assess gas production from feed fermentation, have also been developed.

Menke *in vitro* gas production

Anaerobic digestion of carbohydrates by ruminal microbes produces volatile fatty acids, carbon dioxzide and methane and traces of hydrogen. Therefore measurement of gas production *in vitro* can be used to study the rate and extent of digestion of feedstuffs. Menke *et al.* (1979) developed an *in vitro* gas production technique to estimate feed digestibility. In this method, samples are incubated with rumen fluid with five different solutions (micromineral, buffer, macromineral, resazurin and reducing solutions) under anaerobic conditions at 38°C for 24 hours. Determination of organic matter digestibilities (OMD), ME and Net energy lactation (Nel) are then calculated from gas production in millilitres (Gb), and the crude protein (CP), crude ash (XA), crude lipids (EE) and nitrogen free extractives (NFE) constituents in g/Kg DM as follows:

I.) OMD % = 14.88 + 0.889Gb + 0.045CP + 0.065XA

II.) ME (MJ/Kg DM) = 2.2 + 0.136Gb + 0.0057CP + 0.00029EE^2 (roughages)

III.) NEL (MJ/Kg DM) = 0.0663Gb + 0.095CP + 0.0228EE + 0.077NFE - 3.49
(For milk production food which contains up to 14.9% DF in DM)

IV.) NDL (MJ/Kg DM) = 0.1149Gb + 0.054CP + 0.139EE + 0.54XA - 0.36
(For milk production food which contains 15% CF and more)

The use of gas production to study carbohydrate digestion presents an advantage over the traditional Tilley and Terry gravimetric method because it accounts for both soluble and insoluble substrates (Pell and Schofield, 1993).

In using the gas production, account needs to be taken of the altitude when comparing data from different laboratiories since the volume readings are subject to considerable variation in relation to altitude (Theodorou *et al,* 1994). One of the most serious problems associated with using gas production is that the amount of gas produced varies with different molar proportions of volatile fatty acid production; a higher propionate concentration is associated with lower gas production because an extra carbon atom in propionate would otherwise have appeared as carbon dioxide (Wolin, 1960). It is important to monitor the molar proportions of volatile fatty acids to correct for such differences. Equations to deal with this problem have been proposed by Beuvink and Kogut (1993) and Schofield *et al.* (1994). However, problems of maintaining cannulated animals to provide a source of microbial inoculum also apply to this

In order to avoid problems of gas reading and correction for the different molar proportions of the volatile fatty acids, the *in sacco* technique was developed where assessment of diet degradabilities is conducted within the animal itself.

In sacco (nylon bag) technique

The *in sacco* (nylon bag) technique is a rapid method for determining substrate degradation. The technique involves suspending nylon bags containing different feedstuffs in the rumen for a set period of time or serially removed in order to obtain an estimate of the rate and extent of degradation. The technique incorporates animal and microbial factors helpful in quantifying substrate degradation in the rumen. The *in sacco* degradation, combined with estimated outflow rates, estimates the effective substrate degradation. The 48 hour degradability has been observed to be highly correlated to total tract organic matter digestibility (Barber *et al.*, 1984) and the energy content can be calculated as for the Tilley and Terry procedure (Section 4.1)

Some of the main factors which must be noted when using the nylon bag technique are : the size of the bag, pore size of the material, treatment and preparation of samples, sample size, sample particle size, sample weight to bag area ratio, incubation time, replication, number of bags incubated, the position of the bags in the rumen and the diet of the animal, its species and physiological state. Ørskov *et al.* (1980) and Huntington and Givens (1995) have reviewed these factors.

The major limitations of the *in sacco* technique as pointed out by Ørskov *et al.* (1980) are : Firstly, since the sample is confined within the bag, it is not exposed to any breakdown due to chewing and rumination. Secondly, food would normally be able to leave the rumen once broken down to a suitable particle size. Thirdly, what is being measured is just the breakdown of material to a size small enough to leave the bag and not necessarily a complete degradation to simple chemical compounds. There is also considerable variation in values obtained across laboratories (Madsen and Hvelplund, 1994)

The major disadvantage of this technique is that cannulated animals have to be maintained. Due to animal welfare concerns also, invasive methods will be difficult to justify in the future.

Enzymatic methods

Systems using cellulases have appeared as a result of fungal enzymes becoming commercially available. Many attempts have been made to predict the nutritive value of forages using the enzymatic preparations (Marten and Barnes, 1980; Osbourn and Siddons, 1980; Jones, 1986). Treatment with cellulase has been preceded or succeeded by other treatments (Van der Meer, 1983).

The conventional method, which is normally used, is that of De Boever *et al.* (1988) which involves three steps: 1. The sample is treated with pepsin solution in hydrochloric acid for 24 hours at 40°C to remove interfering protein, 2. The

hours at 40°C to remove interfering protein, 2. The same solution is then maintained at 80°C for 45 minutes and lastly 3. Cellulase (from *Trichoderma viride*) is included for 24 hours at 40°C. The insoluble part is dried in a glass filter, weighed and ashed. From the weight loss at ashing, the enzyme solubility of organic matter in the dry matter is calculated taking into account the dry matter and ash content (XA) of the sample.

Ebztne solubility of organic matter (ESOM) is calculated as :

% ESOM = %DM-%XA-%G

Where % G is the percentage weight loss at ashing. Enzymatic digestible organic matter (EDOM) is obtained as 10 x ESOM

Starch equivalent (StE) and net energy for lactation (NEL) are then calculated using the following two equations, respectively :

1. StE/Kg = g EDOM x 0.3098 + g fat (with HCL-hydrolysis) x 1.997 - g fibre x 0.307 + g NFE x 0.152 - g ash x 0.794 + g DM x 0.446
2. NEL (MJ/Kg DM) = g EDOM x 0.00362 + g fat (with HCL-hydrolysis) x 0.0295 + g protein x 0.0060 + g NFE x 0.0086 - g DM x 0.00235

An advantage of the cellulase method is that problems of maintaining cannulated animals and anaerobiosis are avoided. The method is relatively cheap, simple (since all operations are carried out in one container), fast and there is less contamination of feed residue. The results are also highly repeatable.

The current problems associated with the use of the cellulase method is that the enzymes lack the ability of living organisms to adapt to a substrate and the quality of the cellulase sources has been variable. Enzyme systems are also currently limited by the completeness of the enzyme component. These factors need to be improved if the technique is to find wider application. However, of major importance is that the technique as currently used is highly comparable to the Tilley and Terry, Gas production and *in sacco* Nylon bag techniques.

COMPARISON OF THE CHEMICAL AND *IN VITRO* METHODS AND CONCLUSIONS

The failure to predict exactly *in vivo* digestibilities from chemical or *in vitro* results reflects not only the inherent analytical errors in the methods, but also the fact that *in vivo* digestibility is not a constant characteristic of a feed. It varies according to whether cattle or sheep are used in the trials, the age and health status of the animals, the dietary composition and feeding levels and the manner of feed preparation. Environmental conditions also affect the results obtained. This therefore calls for a general standardisation of the *in vivo* digestibility assessment first as it can confound the comparisons of correlations obtained across and within laboratories with the *in vitro* systems.

From a survey of results of experiments done on the different chemical and *in vitro* techniques, a comparison of their ability to predict *in vivo* nutrient and organic matter digestibilities and therefore energy content can be conducted. Table 1 presents a summary of these results. The following observations can be drawn from this Table:

Table 1 Comparison of the accuracy in the prediction of *in vivo* organic matter digestibility (OMD; dependent variable) for ruminants using chemical and *in vitro* methods as independent variables.

Feed Category	N	Independent variable	Residual standard deviation	Reference
Grass,	18	Crude fibre	2.9	Steg 1981
Grass hay		NDF/ADF/ADL	2.3-3.0	
Grass silage		Tilley and Terry	1.8	
Compound feeds, Compound feed	44	Crude fibre	7.3	Steg 1981
ingredients, Miscellaneous		NDF/ADF/ADL	6.8	
		Tilley and Terry	2.6	
Grass + Hay	19	Tilley and Terry	2.8	Steg *et al.*, 1990
		Pepsin-cellulase	2.1	
Grass	11	Tilley and Terry	1.6	Steg *et al.*, 1990
		Pepsin-cellulase	1.4	
Grass silage	16	Tilley and Terry	1.9	Steg *et al.*, 1990
		Pepsin-cellulase	1.8	
Fodder maize	15	Tilley and Terry	2.2	Steg *et al.*, 1990
		Pepsin-cellulase	1.9	
Compound feed	15	Tilley and Terry	2.5	Steg *et al.*, 1990
		Pepsin-cellulase	2.0	
Compound feed ingredients	60	Tilley and Terry	3.6	Steg *et al.*, 1990
		Pepsin-cellulase	5.5	
Silage	72	Tilley and Terry	2.97	Mannerkorpi
	72	One stage Tilley & Terry	2.21	*et al.*, 1993
	62	Gas test	2.13	Mannerkorpi
	72	Cellulase	1.62	*et al.*, 1993
	62	Cellulase	2.87	
Hay	16	NIR	2.17	Mannerkorpi
	16	Tilley and Terry	2.56	*et al.*, 1993
	16	One stage T & T	2.30	
	16	Gas test	1.77	
Straw	15	Cellulase	2.17	Mannerkorpi
	15	Tilley and Terry	2.56	*et al.*, 1993
	15	One stage Tilley & Terry	2.30	
Dry roughages, Cereals, Cereal by- products,	25	Cellulase	0.037	Aufrère and
Legume seeds roots and by-products, Canning		Cellulase (DM)	0.032	Michalet-
by-products, Oil by-products, Fermentation		Cellulase(OM)		Doreau, 1985
by-products				
Composite feeds	13	Pepsin-cellulase	0.033	Aufère and
				Michalet-
				Doreau, 1988
Single feeds	25	Proximate	0.118	Aufère and
		Van Soest ADF	0.087	Michalet-
		Telley and Terry	0.052	Doreau, 1988
		Pepsin-cellulase	0.032	

N = numbr of samples used in the analysis; DM = dry matter; OM = organic matter; ADF = Acid detergent fibre; NDF = Neutral detergent fibre; ADL = Acid detergent lignin; NIR = Near infra-red reflectance spectroscopy.

1. The use of feed chemical constituents in predicting the organic matter digestibility of energy supply is always associated with a high residual standard dveiation of coefficient of variation.
2. The use of the *in vitro* techniques results in lower residual standard deviation or coefficient of variation than that of chemical constituents.
3. Comparison between the two-stage Tilley and Terry and cellulase *in vitro* methods for maize silage, grass silage and hay samples results in similar prediction errors. However, for straw samples, the cellulase technique has been found to be imprecise.
4. The pepsin-cellulase method has been found to rank better than the *in vitro* Tilley and Terry procedure for predicting the organic matter digestibility of single by-product feeds.
5. In the case of compound feeds for dairy cattle, the pepsin cellulase method and the Hohenheim gas test were similar in the estimation of energy contents. However, for the evaluation of raw materials, the gas test has been found to be superior to the pepsin-cellulase method.

Therefore for a range of feedstuffs for which comparisons could be obtained, the pepsin-cellulase gave poor predictions only in cases of straws and raw materials for dairy feeds. In the case of other by-products, complete feeds, silages and hays, the pepsin-cellulase method provides comparably very good predictions. No comparisons of the pepsin-cellulase method with the nylon bag could be obtained. However, since results of nylon bag degradation are highly correlated to those of *in vitro* gas production (Blümmel and Ørskov, 1993), results of the gas production can be taken as being representative of the nylon bag.

Table 2 presents a comparison of the four feed evaluation techniques to predict digestibility in terms of the requirements, technical features and relative cost of analysis. In terms of requirements, the cellulase method ranks lowest compared to the nylon bag, gas prodution and the Tilley and Terry methods. It is the only method, which combines features of high precision, simplicity and rapidity though standardisation has been found to be problematic. The cost of the analysis is drastically reduced because maintenance of cannulated animals is not necessary.

Table 2 Comparison of the four feed-evaluation techniques to predict digestibility in terms of the requirements, technical features and relative cost of analysis. Modified from that of Osuji *et al.* (1993)

Feature	Tilley and Terry	Gas Production	Nylon bag	Cellulase
1. Requirements				
a. Incubator	Yes	Yes	No	Yes
b. Electricity	Yes	Yes	Yes[U]	Yes
c. Chemicals for buffer	Yes	Yes	No	Yes
d. CO_2 tank	Yes	Yes	No	No
e. Fistulated animals	Yes	Yes	Yes	No
f. Relative labour needs	Low	Low	Low	Low
2. Technical features				
a. Relative precision	High	Low	Low	High
b. Ease of standardisation	Easy	Easy?	Difficult	Difficult
c. Estimate rate of digestion	Yes	Yes	Yes	No
d. Estimate extent of digestion	Yes	Yes	Yes	Yes
e. Relative number of samples/batches	High	High	Low	High
f. Simplicity	Difficult	Difficult	Simple	Simple
g. Time duration	Lengthy	Rapid	Lengthy	Rapid
3. Relative cost of analysis				
a. Instrument	High	High	Low	High
b. Chemicals	High	High	Zero	High
c. Labour, laboratory technician	Low	Low	Low	Low
d. Feed, labour for fistulated animals	High	High	High	Zero
e. Other materials (glassware etc.)	High	High	Low	High

[U] For sample processing.

Therefore, considering cost, precision, rapidity and simplicity, the pepsin-cellulase method holds promise as a routine method of feed analysis. In addition, the method is also non-invasive. More effort should be targeted in developing enzyme mixtures to cover a wider range of feed resources to improve the applicability of the technique.

REFERENCE

Aufère, J. and B. Michalet-Doreau. 1985. *In vivo* digestibility and prediction of digestibility of some by-products. Feeding value of by-products and their use by beef cattle, EEC Seminar, Gontrode, September 27-29.

Aufère, J. and B. Michalet-Doreau. 1988. Comparison of methods for predicting digestibility of feeds. Anim. Feed Sci. and Techno1. 20:203-218.

Ausschuß für Bedarfsnormen der Gesellschaft für Ernährungshpysiologie, 1991. Leitlinien für die Bestimmung der Verdaulichkeit von Rohnährstoffen an Wiederkäuern. J. Anim. Physiol. Anim. Nutr. 65:229-234.

Barber, W.P.B; A.H. Adamson and J.F.B. Altman. 1984. New methods of feed evaluation. In: Recent Advances in Animal Nutrition - 1984, Deitors, Haresign. W. and Cole, D.J.A., pp 161-176, Butterworths, London.

Beuvink, J.M.W. and J. Kogut. 1993. Modelling gas production kinetics of grass silages incubated with buffered ruminal fluid. J. Anim. Sci. 71:1041-1046.

Cone, J.W; Cline-Theil, W; A. Malestein and A.T. van't Klooster. 1989. Degradation of starch by incubation with rumen fluid. A comparison of different starch sources. J. Sci. Food Agric. 49:173-183.

De Boever, J.L; B.G. Cottyn; J.I. Andries; F.X. Buysse and J.M. Vanacker. 1988. The use of a cellulase technique to predict digestibility, metabolisable and net energy of forages. Anim. Feed Sci. Technol 19:247-260.

De Boever, J.L; B.G. Cottyn; D.L. Brabander; J.M. Vanacker and Ch.V. Boucqué. 1996. Equations to predict digestibility and energy value of grass silages, maize silages, grass hays, compound feeds and raw materials for cattle. Agricultural Research Centre - Ghent. National Institute for Animal Nutrition, Communnication No. 911, 42 pp.

Huntington, J.A. and D.I. Givens. 1995. The *in situ* technique for studying the rumen degradation of feeds : A review of the procedure. Nutr. Abstr. Rev. (Series B). 65(2) : 63-93.

Jones, D.I.H. 1986. Cellulase based methods for evaluating forage quality. Bull. Intern. Dairy. Fed. 196 : 33-35.

Madsen, J. and T. Hvelplund. 1994. Prediction of *in situ* protein degradability in the rumen. Results of a European ring-test. Liv. Prod. Sci. 39 : 201-212.

Mannerkorpi, P.; M. Suvitie; M. Touri and T. Heikkilä. 1993. Ein Vergleich fünf verschiedener *in vitro* Methoden zur Schätzung des Futterwertes von Grundfutter. Proc. Soc. Nutr. Physiol. 2 : 11.

Marten, G.C. and R.F. Barnes. 1980. Prediction of energy digestibility of forages with *in vitro* rumen fermentation and fungal enzyme systems. In : Pigden, W.J., Balch, C.C: and Graham, M.(Editors). Standardisation of Analytical Methodology for Feeds. International Development Research Centre, Ottawa, Canada, 128 pp.

Menke, K.H; L. Raab; A Salewiski; H. Steingass; D. Fritz and W.Schneider. 1979. The estimation of digestibility and metabolisable energy content of

ruminant feedstuffs from the gas production when they are incubated with rumen liquor *in vitro*. J. Agric. Sci. Camb. 193 : 217-225.

Ørskov, E.R. and I. McDonald. 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.

Ørskov, E.R.; F.D. DeB. Hovell and I. Mould. 1980. The nylon bag technique for nutritional studies in ruminants. Trop. Anim. Prod. 5 : 195-205.

Osbourn, D.F. and R.C. Siddons. 1980. Enzymatic methods to predict the value of the energy and protein in feeding stuffs. Ann. Zootech. 19 : 325-336.

Osuji, P.O; I.V. Nsahlai and H. Khalili. 1993. Feed evaluation ILCA Manual 5. ILCA (International Livestock Centre for Africa), Addis Ababa, Ethiopia. 40 pp.

Pell, A.N. and P. Schofield. 1993. Computerised monitoring of gas production to measure forage digestion *in vitro*. J. Dairy Sci. 76 : 1063-1073.

Schofield, P; R.E. Pit and A.N. Pell. 1994. Kinetics of fibre digestion from *in vitro* gas production. J. Anim. Sci. 72 : 2980-2991.

Steg, A. 1981. Voorspelling van voederwaarde met laboratoriumtechnieken. IVVO-rapport 140 bijdr. 3.

Steg, A; S.F. Spoelstra; J.M. van der Meer and V.A. Hindle. 1990. Digestibility of grass silage Neth. J. Agric. Sci. 38 : 407-422.

Theodorou, M.K; B.A. Williams; M.S. Dhanoa; A.B. McAllan and J. France. 1994. A simple gas production method using a pressure transducer to determine fermentation kinetics of ruminant feeds. Anim. Feed Sci. Technol. 48 : 185-197.

Tilley, J.M.A. and R.A. Terry. 1963. A two stage technique for the *in vitro* digestion of forage crops. J. Br. Grssld Soc. 18 : 104-111.

Van der Meer, J.M. 1983. European *"In vitro"* ring test 1983. Statistical report 155 of the Institute for Livestock Feeding and Nutrition Research, Lelystad, The Netherlands, 36 pp.

Van Soest, P.J. 1982. Nutritional ecology of the ruminant. O & B Books, Oregon (USA), 374 pp.

Wolin, J.J. 1960. A theoretical rumen fermentation balance. J. Dairy Sci. 43 : 1452-1459.