In vitro estimation of rumen fermentable organic matter using enzymes

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Abstract

The amount of rumen fermentable organic matter (FOM) can be calculated using tables, taking into account the amount of digestible organic matter, the content of fat and fermentation products, and the amount of starch and protein escaping rumen fermentation, or FOM can be calculated using *in situ* incubations. In this paper an *in vitro* method is described to predict FOM using amylase and other carbohydrate degrading enzymes. FOM determined with the enzymatic method showed a moderate correlation ($R^2 = 0.71$) with FOM determined with the *in situ* method. The relationship could be improved by separating the high crude fibre samples ($R^2 = 0.88$) from the other samples ($R^2 = 0.77$). Because degradation rates with the enzymatic method were high compared to the assumed rumen passage rates, it proved that FOM could be predicted with a similar accuracy ($R^2 = 0.76 - 0.80$) by the undegraded fraction after 24 h.

Introduction

In the Dutch protein evaluation system for ruminants, the DVE/OEB-system, (Tamminga *et al.*, 1994), the protein value of feedstuffs is evaluated as the amount of true protein truly absorbed from the small intestine (DVE). This protein originates from feed protein escaping from rumen fermentation (EP), and microbial protein synthesized in the rumen with a correction for protein required to compensate for endogenous losses. In the DVE/OEB-system microbial protein synthesis in the rumen depends on available energy, expressed as the amount of fermentable organic matter (FOM). In the DVE/OEB-system a constant ratio between FOM fermented into volatile fatty acids and FOM incorporated in microbial organic matter is assumed, resulting in a fixed microbial protein yield kg⁻¹ FOM.

In the DVE/OEB-system, FOM is estimated from digestible organic matter corrected for crude fat, EP, escape starch and organic acids. For many feedstuffs values can be obtained from feeding tables (e.g. Vérité *et al.*, 1979; Madsen, 1985;

Anonymous, 1992). Alternatively, the amount of FOM can be estimated from the disappearance of organic matter (OM) measured with *in situ* incubations (Mehrez & Ørskov, 1977; Robinson *et al.*, 1986). Recently, two *in vitro* methods were described to determine FOM, using fresh rumen fluid and a cell free preparation of rumen fluid (Cone *et al.*, 1994). Both *in vitro* methods gave a good prediction of the *in situ* determination of FOM for the majority of the feedstuffs. However, for some feedstuffs, rich in starch or fat, the correlation was poor. In this paper, an *in vitro* method is described to predict FOM using amylase and a carbohydrate degrading enzyme preparation.

Materials and Methods

Feed samples

The chemical composition of 18 feed samples used and their FOM-contents, estimated either according to the DVE/OEB-system, from *in situ* results or from *in vitro* incubations, using fresh rumen fluid or a cell free preparation of rumen fluid, have been reported previously (Cone *et al.*, 1994). For this reason full results of these 18 samples are given in this paper to make comparison with the previous results (Cone *et al.*, 1994) possible. In addition to these 18 samples, the enzymatic method was tested with another set of 91 samples with known *in situ* OM degradation, consisting of maize silage (29 samples), concentrates (16), grass (14), grass silage (12), clover (8), chopped ear corn silage (CEC, 6), and corn cob mix (CCM, 6). These samples were previously used in other experiments in our institute, dried at 70°C and stored at room temperature. However, 7 grass and 6 grass silage samples were freeze dried.

The *in situ* data from the first 18 samples were reported by Cone *et al.* (1994). The *in situ* measurements of the other 91 samples were carried out similarly as described by Cone *et al.* (1994), according to the method described by Van Vuuren *et al.* (1989).

In vitro measurements using enzymes

All samples were ground to pass a 1 mm screen. The feed samples were incubated in 50 ml 0.1 M citric acid/Na-phosphate buffer (pH 5.5) at 39°C in 100 ml centrifuge tubes, which were shaken frequently. The buffer and pH were chosen because the used enzyme preparations proved highest activity under these conditions. The 18 feed samples (0.5 g, dried at 70°C) were incubated for 0, 2, 6, 8, 24, 32, and 48 h, whereas the 91 samples were incubated for only 0, 2, 6, and 24 h. The used buffer contained per litre 1 ml Viscozyme (NOVO 120L, act. 120 FBG/g, Novo Nordisk, Bagsvaerd, Denmark) and 1 ml Termamyl (NOVO 120L, act. 120 KNU/g). Viscozyme is a multi-enzyme complex of *Aspergillus sp*, containing a wide range of carbohydrases, including arabinase, cellulase, ß-glucanase, hemicellulase and xylanase. Termamyl is a heat stable α-amylase from *Bacillus licheniformis*. After the incubation, the solution was filtered and washed by suction through a P1 glass filter in the presence of seasand. The residue on the filter was dried (4 h, 103°C),

weighed, ashed (2 h, 550°C) and weighed again. It is assumed that a non-linear degradation of the OM occurs (Ørskov and McDonald, 1979), as shown by equation (1), from which the degradation rate (k_d) can be calculated.

$$OM\text{-residue} = U + D * e^{(-kd * t)} \qquad (1)$$

In this equation OM-residue is the residue of OM at time t, U is the undegradable fraction and D is the potential degradable fraction (1000 − soluble fraction (S) − U). The OM fraction disappeared after 0.5 h of incubation without added enzymes, was regarded as the soluble fraction (S). The OM residue after 24 h incubation was regarded as U (U-enzyme) for the 91 samples, whereas for the 18 samples the asymptotic lowest level of OM residue after fitting the data with equation (1) was regarded as U-enzyme. FOM was estimated using values of the rumen passage rate (k_p) of 0.060 h^{-1} for concentrates and 0.045 h^{-1} for roughages (Vérité et al., 1987). All incubations were done at least in duplicate.

Results and discussion

Table 1 presents the soluble fraction (S), the undegradable fraction (U), the degradable fraction (D), the degradation rate (k_d), and FOM-values of the 18 feed

Table 1. Fermentable organic matter (FOM) determined with an enzymatic method. S = soluble fraction (g kg^{-1} OM), U = undegradable fraction (g kg^{-1} OM), D = degradable fraction (g kg^{-1} OM); k_d = degradation rate in h^{-1}, FOM = fermentable organic matter (g kg^{-1} OM), SEM = standard error of the mean for FOM. se = solvent extracted, CCM = corn and cob meal, CEC = chopped ear corn.

Feedstuff	Fraction (g kg^{-1} OM)			kd	FOM	SEM
	S	U	D			
Grass A	187	753	60	0.058	197	2
Grass silage	256	677	67	0.036	253	3
Grass B	275	575	150	0.071	323	9
Clover A	248	510	242	0.150	385	2
Maize silage	196	524	280	0.248	416	19
Clover B	272	393	335	0.254	486	3
Soybean hulls	111	637	253	0.139	272	3
Breweries grains, silage	79	821	100	0.086	133	11
Breweries grains, fresh	88	790	122	0.055	141	19
Palmkernel expeller	75	808	117	0.047	120	3
Maize glutenfeed	262	489	248	0.120	399	10
Hominy feed	241	438	321	0.144	445	15
Concentrate	312	401	287	0.154	465	19
Soybean meal, se	244	376	381	0.101	446	1
Potato pulp	167	382	451	0.137	469	9
Sugarbeet pulp	389	182	429	0.225	666	7
CEC-silage	178	289	533	0.207	578	25
CCM	135	243	622	0.164	579	16

samples as obtained with the *in vitro* method using enzymes. The S-values of CCM and CEC-silage were considerably lower than when using the *in situ* method (Cone *et al.*, 1994), where S was determined by washing unincubated bags in a washing machine, causing a loss of starch granules being smaller than the pore size of the nylon bags (41 µm). The upper 6 feeds in Table 1 were regarded as being roughages (k_p of 0.045 h^{-1}), whereas the lower 12 samples were regarded as being concentrates (k_p of 0.06 h^{-1}) (Vérité *et al.*, 1987). The U-enzyme fraction ranged from 182 to 821 g kg^{-1} OM, which was relatively high compared to the *in situ* results, where U-*situ* ranged from 7 to 217 g kg^{-1} OM (Cone *et al.*, 1994). This automatically implied a relatively low D, compared to the *in situ* technique. The OM degradation with the used enzyme preparations proved very fast during the first hours of incubation, after which the enzyme activity decreased rapidly, reaching the maximum amount of OM degraded already after less than 24 h, being the reason for the relatively high values of U-enzyme compared to U-*situ*. Because of this high initial activity of the enzyme preparations, the calculated k_d (equation 1) was relatively high, ranging from 0.036 to 0.254 h^{-1}, whereas *in situ* the k_d ranged from 0.024 to 0.097 h^{-1} (Cone *et al.*, 1994).

The relationship between the FOM-values obtained with the enzymatic method (FOM-enzyme) and obtained by calculation (FOM-calculated), according to the DVE/OEB-system (Tamminga *et al.*, 1994) for the 18 feed samples was moderate ($R^2 = 0.68$; RSD = 94). This was due to the different ways of determining FOM. The FOM-calculated is an average value and does not take account of properties of individual samples. The relationship between FOM-enzyme and the FOM determined with the *in situ* method (FOM-*situ*) (Cone *et al.*, 1994) for the 18 samples showed a higher correlation ($R^2 = 0.85$, RSD = 52), with the exception of breweries grains, fresh and as a silage, and palmkernel expeller, which were rich in lipids. These samples showed relatively high FOM values with the *in situ* method, probably due to the disappearance of crude lipid from the nylon bags. This problem can be overcome by defatting the samples in advance of the experiments. The enzymatic method gave also a good relationship with FOM-*situ* for the starch-rich samples CCM and CEC, which was not the case comparing FOM-*situ* with the FOM determined *in vitro* using fresh rumen fluid and a cell free preparation of rumen fluid (Cone *et al.*, 1994). The amylase used in excess in the enzymatic method may be responsible for a higher degradation of starch compared to the other *in vitro* methods.

The relationship between FOM-*situ* and FOM-enzyme was validated by testing another set of 91 feed samples. Figure 1 shows results of the 91 samples, combined with the samples shown in table 1, with the exception of the fatty samples breweries grains and palmkernel expeller. The overall R^2 was 0.71 and RSD was 52. The relationship could be improved by dividing the samples into two groups, based on the crude fibre and neutral detergent fibre (NDF) content (more or less than 200 g crude fibre kg^{-1} OM or more or less than 500 g NDF kg^{-1} OM). The high crude fibre group contained grass, grass silage and soybean hulls, while the low crude fibre group consisted of the remaining samples. FOM-*situ* for the high crude fibre group was defined as 1.66 (± 0.12) * FOM-enzyme − 33 (± 37) ($R^2 = 0.88$, RSD = 37), whereas for the other group FOM-*situ* was defined as 1.09 (± 0.07) * FOM-enzyme + 75 (± 31) ($R^2 =$
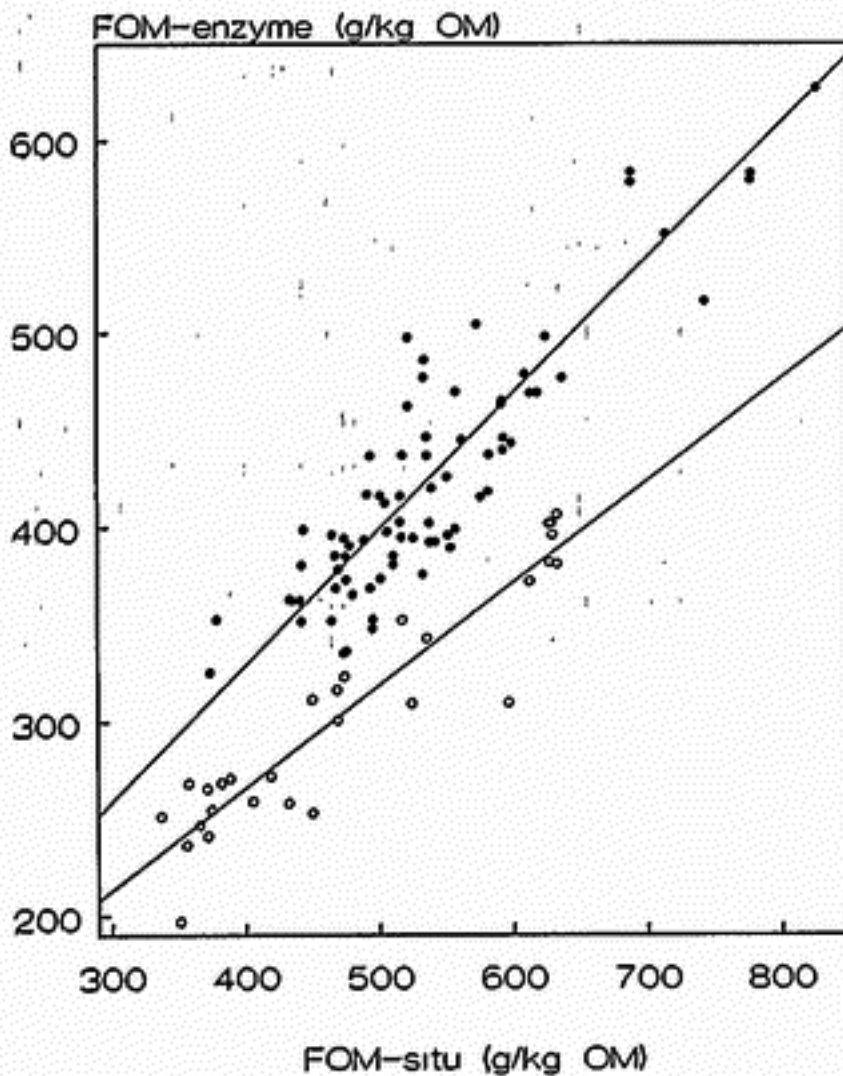
Figure 1. Relationship between rumen fermentable organic matter (FOM) determined with the *in situ* method (FOM-*situ*) and with an enzymatic method (FOM-enzyme). O = grass and grass silage samples, ● = concentrates, maize and clover samples. For the grass samples FOM-*situ* = 1.66 (± 0.12) * FOM-enzyme − 33 (± 37) (R^2 = 0.88, RSD = 37), whereas for the other samples FOM-*situ* = 1.09 (± 0.07) * FOM-enzyme + 75 (± 31) (R^2 = 0.77, RSD = 42).

0.77, RSD = 42). Compared to the other samples, FOM of the high crude fibre group was relatively low using the enzymatic method. The used enzyme preparation proved relatively inactive for samples high in crude fibre compared with the other investigated samples. This can be due to the limited activity and/or lifetime of cellulase and hemicelllulase in the enzyme preparation or to the different pretreatments of the samples before the experiments. *In situ* experiments with nylon bags are usually carried out with fresh materials, which have been frozen and thawed, damaging the cell wall, whereas the *in vitro* experiments were carried out with dried and milled samples. Drying causes a loss of volatile fatty acids, which are not incubated *in vitro*. Drying at 70 °C and freeze-drying cause major differences in N-content of the NDF but only minor differences in fermentation kinetics in rumen fluid (Cone *et al.*, 1996). No differences were obtained in determining FOM after freeze-drying or drying at 70 °C (data not shown).

Although the assumed k_p for roughages and concentrates (Vérité *et al.*, 1987; Madsen, 1985) may be disputable, using different values of k_p does not influence the

ranking of the different samples, because the same k_p was used in the different methods. However, as Table 1 shows, k_d-values were relatively high compared to k_p-values. Consequently, the amount of D contributing to the FOM became nearly 100 %, because the factor $k_d/(k_d + k_p)$ varied between 0.71 and 0.94. Moreover, the proportion of D was low compared to U for the majority of the samples. This means that FOM-enzyme was nearly completely determined by S plus D. Because S + D = 1000 – U, FOM-enzyme can be regarded as a function of U-enzyme. The R^2 for the relationship between U-enzyme after 24 h incubation and FOM-enzyme was 0.97 and the RSD was 21.

Figure 2 shows the relationship between the U-enzyme and FOM-*situ* for all investigated samples ($R^2 = 0.67$, RSD = 56), with the exception of the high fat samples breweries grains and palmkernel expeller. Also here the high crude fibre samples deviated from the other samples. For the high crude fibre samples FOM-*situ* was defined as 1430 (± 92) – 1.48 (± 0.14) * U-enzyme ($R^2 = 0.80$, RSD = 48), whereas for the other samples FOM-*situ* was defined as 937 (± 26) – 0.81 (± 0.05) * U-enzyme
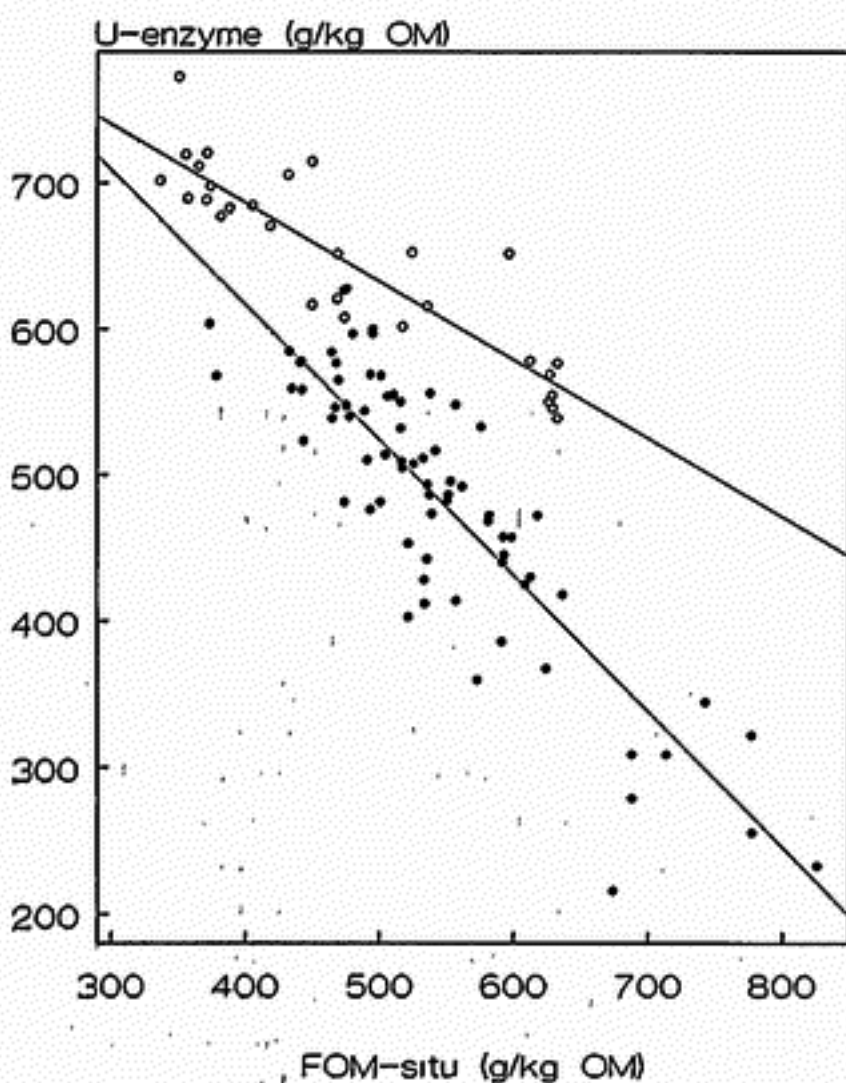

Figure 2. Relationship between rumen fermentable organic matter (FOM) determined with the *in situ* method (FOM-*situ*) and the undegradable fraction after 24 h incubation with enzymes (U-enzyme). O = grass and grass silage samples, ● = concentrates, maize and clover samples. For the grass samples FOM-*situ* = 1430 (± 92) – 1.48 (± 0.14) * U-enzyme ($R^2 = 0.80$, RSD = 48), whereas for the other samples FOM-*situ* = 937 (± 26) – 0.81 (± 0.05) * U-enzyme ($R^2 = 0.76$, RSD = 43).

($R^2 = 0.76$, RSD = 43). This means that FOM-*situ* can be predicted with the same accuracy by determining only the U-enzyme as by determining FOM-enzyme. Moreover, determining the U-enzyme after 24 h incubation is much more easy to perform than all other *in vitro* and *in situ* methods. Another advantage is that this technique uses commercially available enzyme preparations instead of rumen fluid or rumen fluid based enzyme preparations (Cone *et al.*, 1994).

The high values of U and the fast degradation of D shown with the enzymatic method, resulting in relatively high values of k_d, was caused by the nature of enzymes. Microorganisms show a slower degradation, resulting in lower values of k_d (Cone *et al.*, 1994). Microorganisms grow during the incubation and stay active during the entire incubation (low value of U), whereas enzymes have a limited duration of activity, which gradually decreases, resulting in high values of U. Probably no enzyme preparations will become available which can mimic exactly the properties of rumen fluid. The most comparable enzyme preparation to rumen fluid is a cell free preparation of rumen fluid itself. However, the determination of FOM with a cell free rumen fluid preparation (Cone *et al.*, 1994) was not better than with enzymes.

Also for this enzymatic method, the real validation of the results can only be carried out by comparison with *in vivo* data. However, *in vivo* data are scarce and difficult to obtain. Moreover, *in vivo* data can only be obtained from complete rations and it is impossible to obtain them from individual feed ingredients.

It is concluded that there is a good relationship between FOM values obtained with the *in situ* method and with the enzymatic method, after deviation of the feed samples into two groups, based on the crude fibre and NDF content. However, similar results were obtained by determination of U-enzyme after 24 h of incubation. Because the determination of this residue is relatively simple, this method is to be preferred.

Acknowledgements

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