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In vitro estimation of rumen fermentable organic matter using rumen fluid and a cell free preparation of rumen fluid

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Abstract

The amount of protein available for ruminants in the intestine is composed of escape feed protein and microbial protein, synthesized in the rumen. The amount of microbial protein leaving the rumen is considered as a function of the amount of rumen fermentable organic matter (FOM) in the rumen. The 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 ruminal fermentation, or the FOM can be calculated using *in situ* incubations. Both methods have their own disadvantages.

In vitro methods to determine the amount of FOM, using fresh rumen fluid or a cell free preparation of rumen fluid were developed and results were compared with the *in situ* method and the method using the chemical composition of the feedstuff. The *in vitro* methods gave a good prediction of the *in situ* determination of the FOM for the majority of the feedstuffs. However, for some feedstuffs, rich in starch or fat, the correlation was poor. Because no *in vivo* data of FOM were available, it could not be stated whether either the *in vitro* or *in situ* methods gave false results. However, arguments suggest that the *in situ* method is not correct for some feedstuffs.

Keywords: cell free preparation, fermentable organic matter, *in vitro*, *in situ*, microbial protein, rumen fluid.

Introduction

In 1991 a new protein evaluation system for ruminants (DVE ≈ digestible protein in the intestine) was introduced in the Netherlands, in order to optimize protein utilisation and to reduce nitrogen excretion and environmental pollution (Anonymous, 1991). In the DVE system the protein value of feedstuffs is evaluated as the amount of true protein that is absorbed from the small intestine and available for maintenance, growth, gestation and lactation. Protein in the small intestine originates from feed protein escaping rumen fermentation (escape protein, EP), microbial protein synthesized in the rumen and endogenous protein. If nitrogen and other building blocks are not deficient, the DVE system assumes that microbial protein synthesis

depends on available energy, usually expressed as the amount of organic matter (OM) fermented in the rumen. Actually the amount of microbial protein synthesized in the rumen depends on several factors, such as feed composition (Demeyer & Van Nevel, 1986) and microbial turnover in the rumen, largely determined by protozoal predation on bacteria (Demeyer & Tamminga, 1987). In the DVE system the amount of fermentable OM (FOM) is estimated by equation (1):

$$FOM = DOM - CL - EP - ES - 0.5*FP \quad (1)$$

Where
- DOM = digestible OM (g kg^{-1} DM)
- CL = crude lipid (g kg^{-1} DM)
- EP = escape protein (g kg^{-1} DM)
- ES = escape starch (g kg^{-1} DM)
- FP = fermentation products (g kg^{-1} DM)

All parameters involved have to be determined or calculated by other equations. Under practical circumstances FOM can also be estimated using feeding tables (eg Vérité *et al.*, 1979; Madsen, 1985; Anonymous, 1992). However, these tables present average values and escape starch (ES) and EP are based on *in situ* incubations.

The amount of FOM can also be estimated from the OM disappearance measured with *in situ* incubations (Mehrez & Ørskov, 1977; Robinson *et al.*, 1986), using equation (2).

$$FOM = \{S + D \times (k_d/(k_d + k_p))\} \times OM/1000 \quad (2)$$

Where
- S = Soluble OM fraction, disappearing after washing without rumen incubation, expressed in g kg^{-1} DM.
- D = 1000 – S – U (D = degradable fraction, U = undegradable fraction, estimated as the OM residue after 14 days incubation in the rumen). Both D and U are expressed in g kg^{-1} DM.
- k_d = Rate of disappearance of fraction D, expressed in h^{-1}.
- k_p = Passage rate, assumed at 0.060 h^{-1} for concentrates and 0.045 h^{-1} for roughages (Vérité *et al.*, 1987).
- OM = Organic matter, expressed in g kg^{-1} DM.

The advantage of this *in situ* method is that each individual sample can be analyzed, but the method is laborious, expensive, requires specially equipped animals and repeatability may be low (Michalet-Doreau & Ould-Bah, 1992; Nocek, 1988; Van der Koelen *et al.*, 1992). So there is a need for a quick and reliable laboratory method to determine FOM. Existing *in vitro* methods, such as the Tilley and Terry method (Tilley & Terry, 1963), all are end point measurements. In this paper *in vitro* methods with different incubation times using fresh rumen fluid and a cell free preparation of rumen fluid are described. Results were used to estimate FOM which were compared with estimations based on the feeding tables and on *in situ* incubations. The use of a cell free preparation of rumen fluid was earlier described by Cone (1991) simulating *in vitro* rumen degradation of starch.

Materials and methods

Chemical analysis

Feedstuffs were ground to pass a 1 mm screen. Dry matter (DM) content was determined after 4 h at 103 °C and ash after 3 h at 550 °C. Nitrogen (N) was determined using the Kjeldahl method and crude protein (CP) was calculated as 6.25 * N. Crude lipid (CL) was determined by extraction with petroleum-ether (40/60) and starch was determined after Bergmeyer (1970). Neutral detergent fibre (NDF) and crude fibre (CF) were determined after Goering and Van Soest (1970).

In situ *measurements*

The *in situ* OM degradation was determined, according to the method described by Van Vuuren *et al.* (1989). The OM disappearance of the feed samples (c. 5 g DM) was determined after 3, 6, 12, 24, 48 and 336 h. The soluble fraction (S) was determined after a washing program with cold tap water in a washing machine during 45 minutes. Residues at different times of incubation were fitted by a first-order degradation model, including an undegradable fraction (U) and an insoluble, potentially degradable fraction (D), degraded at a constant rate (k_d) (Robinson *et al.*, 1986). The residue after 336 h was considered as U.

Escape protein and starch and DOM

The amount of protein and starch, escaping ruminal fermentation (escape protein; EP, and escape starch; ES), was calculated from the nylon bag incubations after Ørskov & McDonald (1979), using k_p values of 0.060 h^{-1} for concentrates and 0.045 h^{-1} for roughages. The amount of digestible OM (DOM) was determined after Tilley & Terry (1963), corrected with standards with known *in vivo* DOM.

In vitro *measurements using rumen fluid*

The *in vitro* degradation of OM was determined, using the Tilley & Terry method (Tilley & Terry, 1963), excluding the incubation with pepsin/HCl. Rumen fluid was obtained from two lactating Dutch Friesian cows, equipped with a large rumen canula, 2 h after the morning feeding. The cows were fed with 29 kg ration daily, consisting of 60% concentrate, 20% maize silage and 20% artificially dried grass. Before use, rumen fluid was mixed, strained through muslin and mixed 1 to 4 with a phosphate/bicarbonate buffer (pH 6.7) as described by Tilley & Terry (1963).

Samples of feedstuff (0.5 g) were incubated with 50 ml of the rumen fluid/buffer solution at 39 °C for 0.5, 2, 6, 8, 24, 32, and 48 h in 200 ml centrifuge tubes, shaken frequently. After incubation, rumen fluid was filtered and washed by suction through a P1 glass filter. The residue on the filter was dried (4 h, 103 °C), weighed, ashed (2 h, 550 °C) and weighed again. With the results k_d was calculated and FOM was estimated using the method of Ørskov & McDonald (1979), using values of k_p of 0.060

h^{-1} for concentrates and 0.045 h^{-1} for roughages (Vérité *et al.*, 1987). The OM fraction disappearing after 0.5 h was regarded as S, whereas U was calculated by extrapolation. All incubations were done at least twice in duplicate.

In vitro *measurements using a cell free preparation of rumen fluid*

From the two rumen fistulated cows, 2 litre rumen fluid was taken 2 h after the morning feeding during 5 days. Rumen fluid was strained immediately through muslin and centrifuged at 20.000 g for 20 minutes. The pellet was resuspended in a minimum amount of supernatant and sonified intermittently for 12 * 15 seconds and freeze dried (Cone, 1991). Dried material, equivalent to 50 ml rumen fluid was taken for the incubations in 50 ml phosphate/bicarbonate buffer (pH 6.7) (Tilley & Terry, 1963). To avoid bacterial and fungal growth 1 mg l^{-1} tetracycline (T-3258, Sigma, St Louis, MO, USA) and 10 mg l^{-1} nystatin (N-3503, Sigma, St Louis, MO, USA) was added. Determination of the OM degradability was similar to that with fresh rumen fluid. All incubations were done twice in duplicate.

Results

The chemical composition of the feedstuffs used in this study is shown in Table 1. Soybean hulls contained more crude fibre than the other feed samples. The samples

Table 1. Chemical composition of the samples used. OM = organic matter (dry matter – ash), CL = crude lipid, CF = crude fibre, NDF = neutral detergent fibre, CP = crude protein. All contents are in g kg^{-1} DM. – = not determined. se = solvent extracted, CCM = corn and cob meal, CEC = chopped ear corn.

	OM	CL	CF	NDF	CP	starch
grass A	889	–	253	568	168	–
grass silage	886	37	248	521	222	–
grass B	880	–	217	427	183	–
clover A	887	–	179	354	232	–
maize silage	962	–	–	422	78	284
clover B	873	–	137	220	280	–
soybean hulls	949	27	368	620	139	8
breweries grains, silage	964	114	142	558	265	–
breweries grains, fresh	965	119	142	615	269	–
palmkernel expeller	945	117	200	623	164	–
maize glutenfeed	933	41	78	399	225	191
hominy feed	952	51	69	346	152	337
concentrate	898	30	180	338	195	10
soybean meal, se	925	23	54	123	533	–
potato pulp	977	3	168	312	53	352
sugarbeet pulp	915	7	128	300	122	–
CEC-silage	978	–	–	223	79	553
CCM	980	–	–	148	114	571

palmkernel expeller, wet breweries grains and breweries grains silage contained more than 100 g fat per kg dry matter (DM), while solvent extracted soybeanmeal contained more than 500 g protein per kg DM. Most of the feedstuffs contained no or little starch, with the exception of corn cob meal (CCM), chopped ear corn silage (CEC-silage), hominy feed and potato pulp, showing a considerable content of starch. Somewhat less starch was found in maize silage and maize glutenfeed.

Tables 1 to 5 are divided in two parts. The upper 6 feed samples are regarded as being roughages, whereas the lower 12 samples are regarded as being concentrates. Calculations of rumen fermentable organic matter (FOM) for the roughages were carried out assuming a rumen passage rate (k_p) of 0.045 h^{-1} and for the concentrates of 0.06 h^{-1} (Vérité *et al.*, 1987).

Table 2 shows the digestible organic matter (DOM), crude lipid (CL), fermentation products (FP), escape protein (EP) and escape starch (ES) and the FOM values as calculated according to equation (1). The contents of CL and FP in Table 2, marked with an asterix were not determined but taken from the Dutch Feeding Table (Anonymous, 1992). The FOM values ranged from 341 g kg^{-1} DM in fresh breweries grains to 751 g kg^{-1} DM in sugarbeet pulp.

S, U, D and k_d values obtained and k_p values used *in situ* with nylon bag incubations were used to calculate FOM according to equation (2) and are shown in Table 3. Remarkable was the high S for the samples CCM and CEC-silage. The FOM val-

Table 2. FOM calculated after equation (1) and the DVE-table. DOM = digestible OM (g kg^{-1} DM), FP = fermentation products (g kg^{-1} DM), EP = escape protein (g kg^{-1} DM), ES = escape starch (g kg^{-1} DM), EP and ES were obtained with nylon bag incubations, FOM = fermentable organic matter (g kg^{-1} DM). The contents of CL and FP in Table 2, marked with an asterix were not determined but taken from the Dutch Feeding Table (Anonymous, 1992). SE = solvent extracted, CCM = corn and cob meal, CEC = chopped ear corn.

	DOM	CL	FP	EP	ES	FOM
grass A	622	40*	–	73	–	509
grass silage	656	37	40*	80	–	519
grass B	713	40*	–	58	–	615
clover A	656	35*	–	92	–	529
maize silage	702	25*	96*	26	28	575
clover B	707	35*	–	100	–	572
soybean hulls	778	27	–	60	–	691
breweries grains, silage	588	114	50*	87	–	362
breweries grains, fresh	579	119	50*	94	–	341
palmkernel expeller	662	117	–	74	–	471
maize glutenfeed	802	41	–	63	29	669
hominy feed	809	51	–	67	108	583
concentrate	763	30	–	51	2	680
soybean meal, se	833	23	–	235	–	575
potato pulp	879	3	75	34	95	710
sugarbeet pulp	814	7	45*	33	–	751
CEC-silage	822	35*	70*	16	22	714
CCM	853	35*	70*	16	23	744

Table 3. FOM determined *in situ* using the nylon bag method (equation 2). S = soluble fraction (g kg^{-1} DM), U = undegradable fraction (g kg^{-1} DM), D = degradable fraction (g kg^{-1} DM), k_d = degradation rate in h^{-1}, k_p = rumen passage rate in h^{-1}, FOM = fermentable organic matter (g kg^{-1} DM), SEM = standard error of the mean. se = solvent extracted, CCM = corn and cob meal, CEC = chopped ear corn.

	S	U	D	k_d	k_p	FOM	SEM
grass A	97	217	686	0.035	0.045	351	27
grass silage	229	120	651	0.034	0.045	450	5
grass B	171	108	722	0.047	0.045	474	5
clover A	165	171	664	0.057	0.045	475	28
maize silage	368	176	456	0.026	0.045	516	5
clover B	236	82	683	0.055	0.045	534	11
soybean hulls	158	28	814	0.032	0.06	419	13
breweries grains, silage	241	161	598	0.043	0.06	471	16
breweries grains, fresh	275	181	544	0.052	0.06	510	6
palmkernel expeller	216	149	636	0.071	0.06	530	16
maize glutenfeed	290	23	687	0.049	0.06	557	9
hominy feed	389	35	577	0.032	0.06	562	7
concentrate	327	46	628	0.068	0.06	592	21
soybean meal, se	286	7	708	0.061	0.06	593	9
potato pulp	140	60	800	0.097	0.06	618	12
sugarbeet pulp	416	32	552	0.083	0.06	674	7
CEC-silage	611	67	322	0.024	0.06	688	1
CCM	730	48	222	0.024	0.06	777	2

Table 4. Fermentable organic matter (FOM) determined with an *in vitro* method using fresh rumen fluid. S = soluble fraction (g kg^{-1} DM), U = undegradable fraction (g kg^{-1} DM), D = degradable fraction (g kg^{-1} DM), k_d = degradation rate in h^{-1}, FOM = fermentable organic matter (g kg^{-1} DM), SEM = standard error of the mean. se = solvent extracted, CCM = corn and cob meal, CEC = chopped ear corn.

	S	U	D	k_d	FOM	SEM
grass A	106	80	815	0.023	339	4
grass silage	247	45	709	0.023	431	3
grass B	208	105	687	0.047	492	11
clover A	242	278	480	0.053	445	5
maize silage	170	27	803	0.027	453	17
clover B	267	218	515	0.075	514	9
soybean hulls	115	0	886	0.025	356	7
breweries grains, silage	93	469	438	0.048	277	12
breweries grains, fresh	100	433	467	0.036	266	5
palmkernel expeller	26	133	841	0.029	284	8
maize glutenfeed	261	45	694	0.046	525	12
hominy feed	235	30	735	0.040	504	14
concentrate	308	83	609	0.049	522	8
soybean meal, se	269	103	629	0.070	562	5
potato pulp	150	16	835	0.040	473	17
sugarbeet pulp	384	22	594	0.049	596	13
CEC-silage	185	54	762	0.042	488	5
CCM	181	25	794	0.044	507	4

Table 5. Fermentable organic matter (FOM) determined with an *in vitro* method using cell free rumen fluid. S = soluble fraction (g kg^{-1} DM), U = undegradable fraction (g kg^{-1} DM), D = degradable fraction (g kg^{-1} DM), k_d = degradation rate in h^{-1}, FOM = fermentable organic matter (g kg^{-1} DM), SEM = standard error of the mean. se = solvent extracted, CCM = corn and cob meal, CEC = chopped ear corn.

	S	U	D	K_d	FOM	SEM
grass A	193	689	118	0.045	224	1
grass silage	247	605	148	0.038	279	1
grass B	278	498	224	0.050	348	1
clover A	288	504	208	0.102	383	2
maize silage	204	380	416	0.040	385	7
clover B	345	485	170	0.230	425	6
soybean hulls	144	626	230	0.060	246	2
breweries grains, silage	143	662	195	0.035	207	3
breweries grains, fresh	114	737	149	0.057	180	1
palmkernel expeller	49	0	951	0.009	164	3
maize glutenfeed	342	361	297	0.083	480	4
hominy feed	345	326	329	0.054	477	1
concentrate	340	391	269	0.063	429	2
soybean meal, se	288	372	340	0.034	380	1
potato pulp	184	475	341	0.065	353	4
sugarbeet pulp	431	236	333	0.103	587	2
CEC-silage	294	149	557	0.041	509	9
CCM	252	90	658	0.041	509	2

ues ranged from 351 g kg^{-1} DM in grass A to 777 g kg^{-1} DM in CCM.

Table 4 presents S, U, D, k_d, and FOM values of the different feed samples as obtained with the *in vitro* method using fresh rumen fluid. The S of CCM and CEC-silage was considerably lower than when using the *in situ* method (Table 3), were S was determined after a washing program in a washing machine. There were differences in the different parameters determined, comparing Tables 3 and 4. However this did not necessarily cause differences in FOM.

Table 5 presents S, U, D, k_d, and FOM values of the different feed samples as obtained with the *in vitro* method using a cell free preparation of rumen fluid. Using this method U was considerably higher than using the *in situ* or the *in vitro* method with fresh rumen fluid (Tables 3 and 4). Also S was slightly higher when using the cell free preparation than fresh rumen fluid. Apparently the enzymes in the cell free preparation were more active in the first minutes of incubation than the microorganisms in the fresh rumen fluid, as S was determined after 0.5 h incubation. Because of these higher S and U for the cell free preparation, D was lower. Also the FOM values were lower when the cell free preparation method was used than with the fresh rumen fluid *in vitro* method and the *in situ* method.

Figure 1 shows the relationship between the FOM values obtained with the *in situ* method (FOM-situ) and obtained by calculation using equation (1) (FOM-calculated). According to R^2 (0,36) and RSD (85) for all the feed samples the relationship seemed poor. However, there appeared to be a linear relationship for most of the samples, excluding the samples marked A–D (Figure 1). The samples marked A in

Figure 1 are the samples breweries grains, fresh and as a silage and sample B is palmkernel expeller, which all contained more than 100 g fat per kg DM. Excluding these samples R^2 increased to 0.54 and the RSD decreased to 78. Sample C is soybean hulls, which contained a relatively high amount of CF (368 g kg^{-1} DM) and sample D is solvent extracted soybean meal, which contained a relatively high amount of protein (533 g kg^{-1} DM). Excluding all these samples R^2 became 0.81 and the RSD was 51 and the FOM-situ was defined as 1.17 (± 0.17) * FOM-calculated – 177 (± 108).

The relationship between FOM-situ and the FOM determined with the *in vitro* method with fresh rumen fluid (FOM-fresh) is shown in Figure 2. Overall, R^2 was 0,34 and RSD was 86. Also here it appeared that there was a linear relationship between the two methods for most of the feed samples, excluding the samples marked A–D in Figure 2, with somewhat higher FOM-situ values than FOM-fresh values. This was mainly due to two groups of samples deviating from the line. The samples

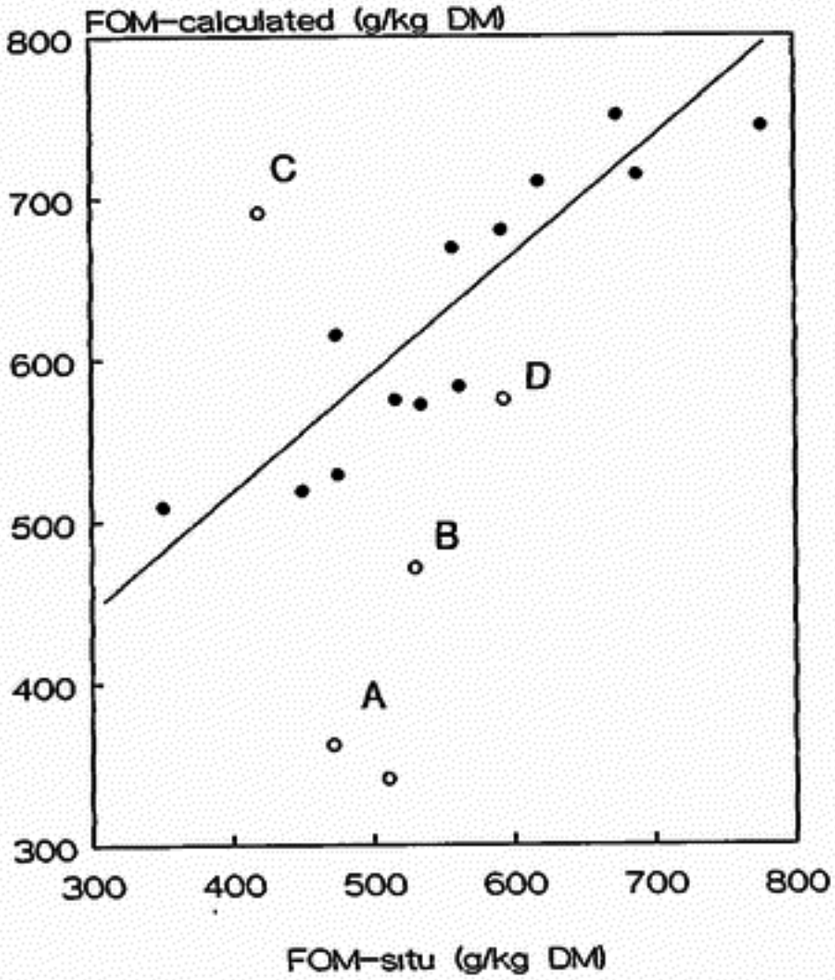

Figure 1. Relation between the amount of rumen fermentable organic matter (FOM) determined with the *in situ* method (FOM-situ) and calculated using equation (1) (FOM-calculated). A = the fatty samples breweries grains, fresh and as a silage, B = palmkernel expeller, C = soybean hulls and D = solvent extracted soybean meal. Using all samples R^2 was 0.36 and RSD was 85. Without the samples marked A, B, C, and D R^2 was 0.81 and RSD was 51 and FOM-situ = 1.17 (± 0.17) * FOM-calculated – 177 (± 108).

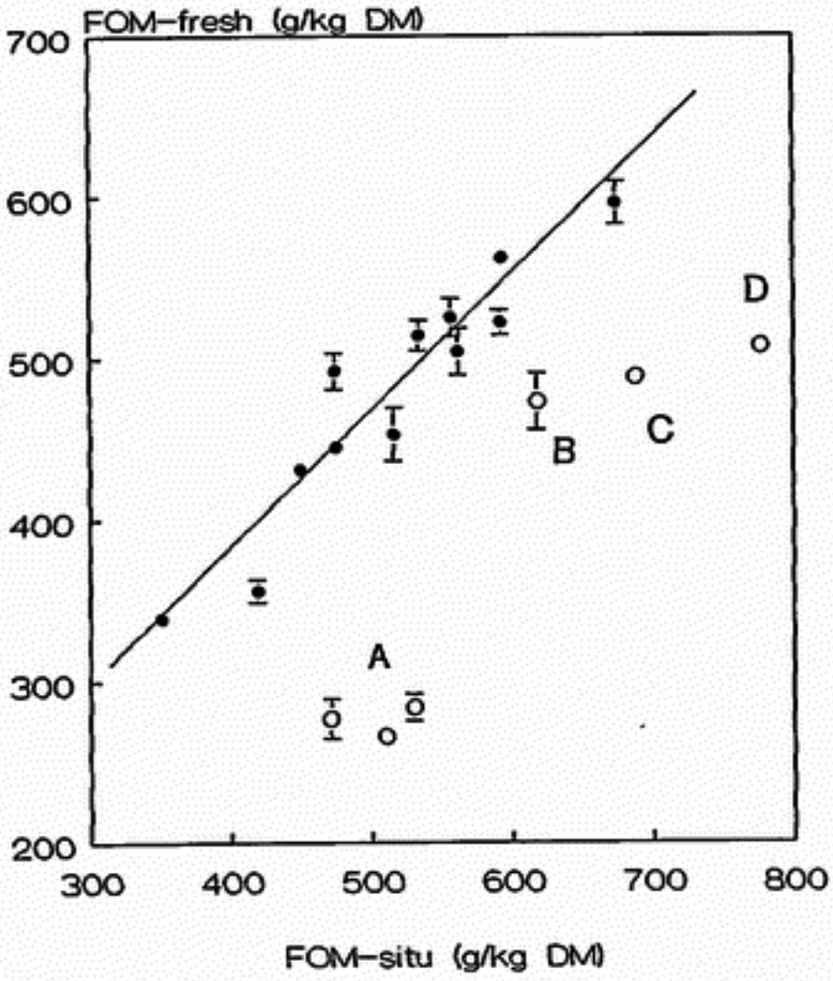

Figure 2. Relation between the amount of rumen fermentable organic matter (FOM) determined with the *in situ* method (FOM-situ) and with an *in vitro* method using fresh rumen fluid (FOM-fresh). A = the fatty samples breweries grains, fresh and as a silage and palmkernel expeller, B = potato pulp, C = CEC-silage and D = CCM. Using all samples R^2 was 0.34 and RSD was 86. Without the samples marked A, B, C, and D R^2 was 0.90 and RSD was 29 and FOM-situ = 1.08 (± 0.11) * FOM-fresh – 2 (± 55).

marked A are the fatty samples breweries grains, fresh and as a silage, and palmkernel expeller. The samples marked B, C and D are the starchy feedstuffs potato pulp, CEC-silage and CCM. Excluding these samples R^2 was 0.90 and the RSD was 29 and FOM-situ was defined as 1.08 (± 0.11) * FOM-fresh – 2 (± 55).

The relationship between FOM-situ and the FOM determined with the *in vitro* method with a cell free preparation of rumen fluid (FOM-cell free) is shown in Figure 3. There was a R^2 of 0,52 and a RSD of 74 for all feed samples. Also here it is shown that there was a linear relationship between the two methods for most of the feed samples (excuding the samples marked A–E in Figure 3), with a considerable higher FOM-situ than FOM-cell free. Also here some samples were deviating from the line. The samples marked A are the fatty samples breweries grains, fresh and as a silage, and palmkernel expeller and the sample marked B is solvent extracted soybean meal which was high in protein content and the samples, marked C, D and E are the starchy samples potato pulp, CEC-silage and CCM respectively. Without these samples the R^2 was 0.92 and the RSD was 27 and the FOM-situ was defined as 0.78 (± 0.08) * FOM-cell free + 208 (± 31).

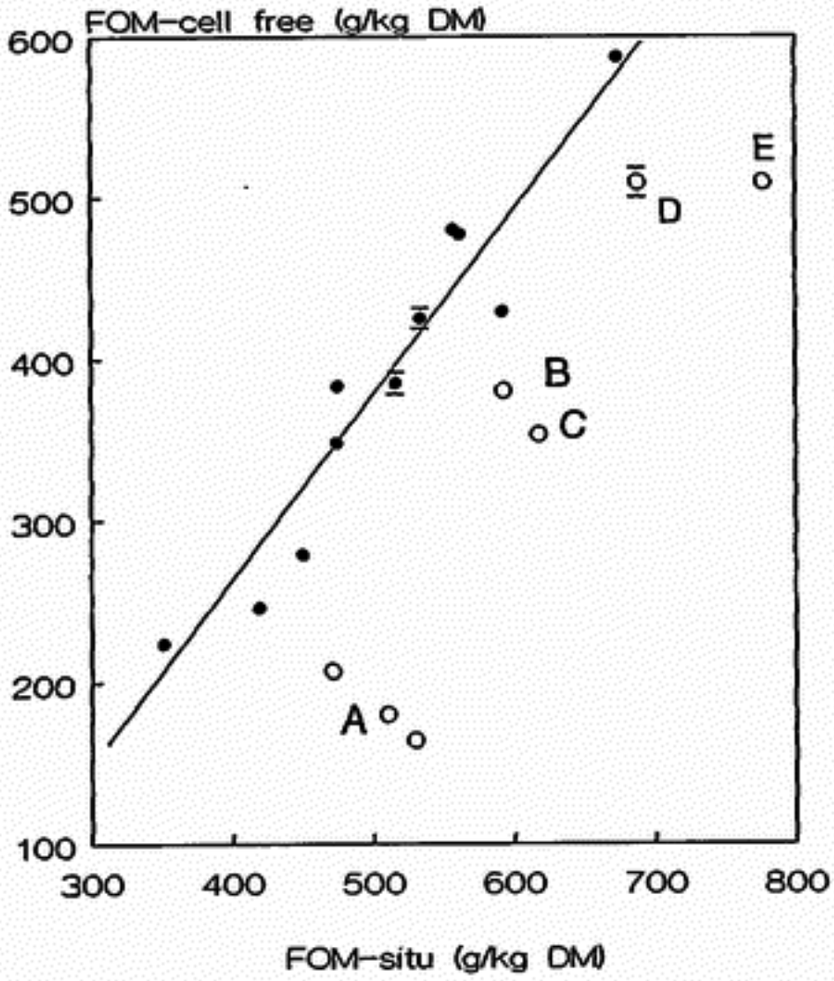

Figure 3. Relation between the amount of rumen fermentable organic matter (FOM) determined with the *in situ* method (FOM-situ) and with an *in vitro* method using a cell free preparation of rumen fluid (FOM-cell free). A = the fatty samples breweries grains, fresh and as a silage and palmkernel expeller, B = solvent extracted soybean meal, C = potato pulp, D = CEC-silage and E = CCM. Using all samples R^2 was 0.52 and RSD was 74. Without the samples marked A, B, C, D, and E R^2 was 0.92 and RSD was 27 and FOM-situ = 0.78 (± 0.08) * FOM-cell free + 208 (± 31).

Discussion

The *in situ* method, using nylon bag incubations, is generally accepted as a method to study degradation kinetics in the rumen (Mehrez & Ørskov, 1977; Lindberg, 1985). However, there are some remarks which can be made on this method (Michalet-Doreau & Ould-Bah, 1992; Nocek, 1988; Van der Koelen *et al.*, 1992). After incubation in the rumen, the nylon bags are washed, using a washing program of 45 minutes in a domestic washing machine, to remove microbial contamination, which is not always complete (Blair & Cummins, 1983; Hof *et al.*, 1990). In the same way S is determined, with nylon bags without rumen incubations at time zero (Cherney *et al.*, 1990; Nocek, 1988; Van Vuuren *et al.*, 1989). Losses of insoluble material are likely, particularly with samples containing small particles (Michalet-Doreau & Cerneau, 1991) or materials may be solubilized but not degraded. The pore size of the nylon bags was 40 µm, which is much larger than the size of starch granules, which is about 10 µm for most of the starches and up to 30 µm on average

for potato starch (Cone & Wolters, 1990). The starch particles disappearing from the bags are regarded as being soluble starch, which is not necessarily the case. Figures 2 and 3 show that the starchy feedstuffs CEC-silage and CCM had a higher FOM content when determined with the *in situ* method than with the *in vitro* methods (FOM-fresh and FOM-cell free). This was largely due to the very high S in these samples obtained in the *in situ* method (Table 3). The FOM of potato pulp was also higher when determined *in situ* than *in vitro*, although S was not significantly different, but the k_d was higher *in situ* than *in vitro*. Probably the potato pulp was more rapidly released from the nylon bags during incubation. The fact that the starch rich feedstuffs were not deviating in the comparison of FOM-situ and FOM-calculated (Figure 1) may be because also in FOM-calculated, escape starch was based on data obtained with nylon bag incubations.

The fate of CL during washing of the nylon bags is uncertain. In equation (1) CL is subtracted from DOM, assuming that the energy supply for microorganisms from CL is negligible. When CL is washed out of the nylon bags, a proportion of CL is regarded as soluble, which means that S and FOM increase. Tables 3, 4 and 5 show that S of the fatty samples, breweries grains and palmkernel expeller with the *in situ* method (Table 3) was relatively high compared to the *in vitro* methods (Tables 4 and 5), resulting in relatively high FOM values for the *in situ* method. Perrier *et al.* (1992) showed that lipids and fatty acids can disappear from nylon bags during rumen incubation (39 °C) up to 70% after 8 h for fat and 95% for fatty acids. After only 1 h even more than 20% of the lipids disappeared from the nylon bags. Our results suggest that fat also disappeared from the nylon bags during the S determination in the washing machine using cold tap water.

There was a poor relationship between the S determined with the *in situ* method and the *in vitro* methods for all the feed samples ($R^2 = 0.06 - 0.11$). Without the samples rich in fat, and CCM and CEC-silage R^2 was increased to 0.45 – 0.47. Because S of the fatty and starchy feed samples, as determined with the *in situ* method, may be too high, it is suggested that also the FOM with the *in situ* method may be overestimated.

Calculation of the FOM with a rumen passage rate (k_p) of 0.045 h^{-1} for roughages and of 0.06 h^{-1} for concentrates, is questionable (Vérité *et al.* 1987; Madsen, 1985). The values of k_p represents an average value of the effective passage rate, including small and large particles (S fraction) and the D and U fractions. A more differentiated use of k_p for different feed components would be preferred. Calculating the FOM with a k_p of either 0.045 or 0.06 h^{-1} causes differences of about 40 to 50 g FOM kg^{-1} DM. However, comparing different methods for the determination of FOM, k_p did not influence the results, because the same k_p was used for the different methods. Differences will only occur when FOM, obtained with the *in situ* and *in vitro* methods, is compared to the calculated FOM, using equation (1). Differences in k_p may also explain the relatively low FOM of soybean hulls estimated by the *in situ* method, compared to the FOM calculated by equation (1) (Figure 1). As stated by Van Vuuren (1993) this higher FOM calculated by equation (1) may be due to an increased ruminal retention time of the high fibre feeds that occurs *in vivo* and increases DOM.

The FOM can be calculated with the *in situ* and *in vitro* incubation data in different ways (Robinson *et al.*, 1986). The U fraction can be calculated by extrapolation or the residue after a long incubation time can be regarded as U. In Tables 3 to 5 the FOM was calculated with an U obtained by extrapolation. However, it proved that there were no considerable differences using the residue after 48 h as the U fraction ($R^2 = 0.997$, RSD = 5).

As an *in vitro* method should be easy to perform and not laborious, the *in vitro* techniques, as described are not very useful, since they make use of too many incubation times. However, comparable results were obtained with only 3 data points at 0.5, 6 and 24 h ($R^2 = 0.99$, RSD = 13) instead of incubation times at 0.5, 2, 6, 8, 24, 32 and 48 h. Using only 3 data points, the 24 h incubation residue was regarded as the U fraction. This makes the *in vitro* technique much more easy to perform. However, both *in vitro* techniques still use rumen fluid and thus fistulated cows are necessary. When there is a possibility to obtain freeze dried cell free rumen fluid in large amounts, this technique is preferable, because of the smaller SEM for the FOM (Table 5). The SEM is smaller because variation in rumen fluid from one day to another day and between animals can be minimized by combining rumen fluid from different animals and during several days. Nevertheless, enzymatic methods to determine the FOM would be preferable. Because, until recently no enzyme preparations were available with amylases, proteases and carbohydrases with pH optima in the same range, no enzymatic methods are yet described.

The results show that there was a good relationship between the FOM determined *in vitro* and *in situ* for several feed samples. The FOM of the fatty and the starchy samples determined *in situ* and *in vitro* did not correlate very well. Both the fatty and the starchy samples showed a higher FOM *in situ* than *in vitro*. Because of the rather rough determination of S with the *in situ* method it is suggested that the FOM for this samples was overestimated with the *in situ* method, rather than underestimated with the *in vitro* methods. This suggest that the *in vitro* methods may give a better approach of the *in vivo* fermentation of OM than the *in situ* method and that in future *in vitro* techniques, which are cheaper, less laborious and more easily reproducible than the *in situ* technique, may be used in feed evaluation systems. However, the real value of *in vitro* techniques can only be obtained after comparison of the *in vitro* and *in sacco* data 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. Mbwile and Udén (1991) also came to the conclusion that the *in situ* method was less reliable and precise than *in vitro* methods comparing *in situ* and *in vitro* data with *in vivo* OM digestibility, possibly as a result of variable microbial environment inside the bags or from uneven extraction during washing of the bags.

Conclusions

It is concluded that there is a good correlation between the FOM obtained with the *in situ* method and with *in vitro* methods for the majority of the feedstuffs tested.

There is no good correlation for feedstuffs high in fat and starch content. It is suggested that the *in vitro* methods came closer to the *in vivo* degradation because of the unphysiological determination of S with the *in situ* methods, showing a too high S for the fatty and starchy feedstuffs. Certainty can only be obtained by comparison of the FOM data with *in vivo* experiments.

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