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Comparison of dry matter and neutral detergent fibre degradation of fibrous feedstuffs as determined with *in situ* and *in vitro* gravimetric procedures

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ABSTRACT

Our goal was to compare two technical approaches (the *in situ* nylon bag technique, IS, and an *in vitro* gravimetric procedure using Daisy^{II}, IV Daisy^{II}) to estimate DM and NDF degradation kinetics of fibrous feedstuffs. Evaluated feedstuffs were four fresh pastures, two high dry matter forages, and two agro-industrial by-products. In the IS, two 2 bags (polyfilament, 5 cm × 9 cm, pore size 50 ± 15 μm, ANKOM 1020) of each feedstuff were incubated (2, 4, 8, 12, 24, 48, 72 and 96 h, 2 consecutive periods) in the rumen of three wethers. In the IV Daisy^{II}, six bags (5 cm × 3 cm, mean pore size 45 μm) of each feedstuff were incubated (same schedule as IS) in digestion jars with a buffer/fluid ruminal solution (80:20). Compared with IS, IV Daisy^{II} underestimated DM and NDF disappearance, and the largest differences between procedures were observed at early incubations times. Both procedures ranked feedstuffs similarly for their DM effective degradability suggesting the IV Daisy^{II} procedure may be a useful tool to compare degradation potential of feedstuffs. Further investigations are needed to study the variations observed in the early incubations times between the two procedures.

1. Introduction

Current feed evaluation systems focus on dynamic aspects of ruminal degradation of dietary constituents (Jarrige, 1989; Sniffen et al., 1992; AFRC, 1993; NRC, 2001). Fibrous feed are the main components of ruminant diets, especially with pasture based systems. In ruminants, variation in fibrous feed digestibility is mainly due to differences in concentration of cell wall carbohydrates (often analyzed as neutral detergent fibre, NDF), and their degradation. The amount of NDF degraded would depend on the relationship between the rate of degradation and the rate of passage of the NDF, and it is influenced by intrinsic characteristics of fibrous feed (Mertens, 1993). It is known that fibre degradation determine physical rumen fill, which is a main factor in ruminant feed intake regulation (Van Soest, 1994).

Abbreviations: *a* fraction, soluble or rapidly degradable fraction; ADF, acid detergent fibre expressed inclusive of residual ash; *b* fraction, insoluble but potentially degradable fraction; CEL, cellulose; CP, crude protein; DIFISIV, difference between mean values of DM and NDF disappearances obtained by the IS and IV Daisy^{II} procedures; DM, dry matter; $ED_{DM/NDF}$, effective degradability of DM or NDF; HEM, hemicellulose; IS, *in situ* procedure; IV Daisy^{II}, *in vitro* Daisy^{II} procedure; *kd*, degradation rate constant; *kp*, ruminal passage rate; *L*, discrete time lag of degradation; lignin (sa), sulfuric acid lignin; aNDF, neutral detergent fibre assayed with a heat stable amylase expressed inclusive of residual ash; NDF, neutral detergent fibre assayed without a heat stable amylase and expressed inclusive of residual ash.

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The nylon or dacron bag technique (IS), is an *in situ* procedure used to evaluate extent and rate of degradation of nutrients in the rumen, and it is considered a reference technique in current feed evaluation systems (AFRC, 1993; Sniffen et al., 1992; Tamminga and Williams, 1998; NRC, 2001). This procedure is time-consuming and labor intensive, and at least three surgically modified animals are required to obtain valid data.

The *in vitro* gravimetric procedure using Daisy^{II} (IV Daisy^{II}, ANKOM Corp., Fairtport, NY, USA) appears to provide highly repeatable disappearance data to estimate both the extent, and rate of degradation of fibrous feed (Mould and Nordheim, 1998; Vogel et al., 1999; Julier et al., 1999; Ceballos et al., 2008). In comparison with the nylon bag technique, this procedure provides considerable savings in terms of fistulated animals, feed, and labor input, as well as in time required to conduct an experiment.

The nylon bag technique and the IV Daisy^{II} procedures were compared by Ceballos et al. (2008) to estimate kinetics of degradation of dry matter (DM) of three tropical grasses (*Shorgum* sp, *Pennisetum purpureum*, and *Pennisetum violaceum*). Bags with larger pore size were used in IS (pore size = 50 μm, R1020; ANKOM Co., Fairport, NY) than in IV Daisy^{II} (pore size = 25 μm, F57; ANKOM Co., Fairport, NY). Authors concluded the techniques were not interchangeable to estimate degradation kinetics, but IV Daisy^{II} predicted with accuracy DM disappearance at 96 h.

In this study, the objective was to compare the nylon bag technique with an *in vitro* gravimetric procedure to estimate DM and NDF degradation kinetics of a wide range of fibrous feedstuffs using bags with similar porosity in both procedures.

2. Materials and methods

2.1. Feedstuffs

Three sets of fibrous feeds (cultivated pastures, high dry matter forages, and agro-industrial by-products) selected to represent a wide range of NDF concentration and composition, were evaluated. Cultivated pastures ($n = 4$) were white clover (*Trifolium repens* sp.), birdsfoot trefoil (*Lotus corniculatus*, va. *San Gabriel*), ryegrass (*Lolium* sp.), and oat (*Avena sativa* sp.); high dry matter forages ($n = 2$) were alfalfa hay, and barley straw, and agro-industrial by products ($n = 2$) were dried brewers' grains, and dehydrated citrus pulp. Pastures samples (legumes in early vegetative, and grasses, 2nd regrowth, vegetative stage) were provided by a Research Project on Nutritional Evaluation of Grazed Forages, alfalfa hay (mid bloom) and barley straw by the Animal Nutrition Laboratory (Faculty of Agronomy, University of the Republic), and by-products were obtained from local agro-industrial plants. Before evaluation, all feedstuffs were dried in an air forced oven at 60 °C, and ground (2 mm) with a Willey mill.

2.2. In situ ruminal degradability

Three Corriedale wethers (LW = 50 kg), fitted with rumen cannula, were used to determine *in situ* (IS) DM and NDF degradation kinetics using the nylon bag technique (Orskov et al., 1980). Animals were housed in individual stalls, and daily fed 1.5 kg of DM of a alfalfa-grass hay (170 and 510 g/kg DM of CP and NDF, respectively) in two equals meals at 8:00 and 16:00 h. Animals had free access to minerals salts (Cobalfosal-Ovinototal, Barraca Deambrosis, Montevideo, Uruguay) and water, and were handled according to the good practices guidance for the Use of Animals in Research, Testing and Teaching of the Universidad de la República of Uruguay.

Polyfilament polyester bags of (ANKOM 1020; size 9 cm × 5 cm; mean pore size 50 μm) containing 1.8 g of DM (15–18 mg/cm^2) were introduced simultaneously in the rumen immediately after the morning meal, and removed sequentially at 2, 4, 8, 12, 24, 48, 72 and 96 h of incubation. Feedstuffs were incubated in the three whethers in two consecutive periods, in order to obtain six disappearance values for each feedstuff/incubation time. Before rumen incubation, bags were submerged (15′) in warm water (39 °C) and after collection from rumen, were soaked in cold water, and stored at −20 °C. Six bags per feedstuff were not incubated in the rumen, and handled similarly to the incubated ones to obtain the zero time incubation (to). Once thawed, bags were washed (three times) with automatic machine (FORTAL XP B30-7S, 30 l capacity, 30 bags per wash cycle, 3 min with program wash soft without centrifugation), dried in a forced air oven (60 °C, 48 h), and weighed. Dry matter losses were computed as the difference in weight of the pre- and post-incubated bags, and expressed as proportion of initial weight. Residues of replicates per time within wethers were pooled prior to analysis.

2.3. In vitro ruminal degradability

In vitro rumen degradability (IV Daisy^{II}) was performed immediately after *in situ* measurements ended, using the Daisy^{II} Incubator (ANKOM Technology Corp., Fairport, NY, USA). Animal donors of rumen fluid were the same as in the IS, and were fed, and handled as described for the IS studies. Bags (5 cm × 3 cm) were made in our laboratory (Animal Nutrition Laboratory, Faculty of Agronomy, University of the Republic, Montevideo, Uruguay) using N-free monofilament polyester clothe (Sefar Inc., Switzerland; 45 μm pore size). Bags containing 0.5 g of sample DM (60 °C) were heat-sealed, and incubated (2, 4, 8, 12, 24, 48, 72 and 96 h) in ruminal fluid combined with a buffer solution (1:4, v/v). Ruminal fluid was collected into bottles (preheated with water at 39 °C) before morning feed and carried immediately to laboratory, where fluids from the three wethers were pooled, strained twice, and mixed with the buffer solution. In the first and second straining, respectively, two and eight layers of cheesecloth were used. The buffer solution was a combination of two buffers (solutions A and B)

as indicated in the ANKOM procedure for *in vitro* true digestibility. Solution A (1330 ml) contained 10 g/l of KH_2PO_4, 0.5 g/l $MgSO_47H_2O$, 0.5 g/l NaCl, 0.1 g/l $CaCl_22H_2O$ and 0.5 g/l of urea (reagent grade), and solution B (266 ml) contained 15 g/l of Na_2CO_3 and 1.0 g/l of Na_2S9H_2O. In each digestion jar, 1600 ml pre-warmed combined buffer solution was added; then, jars were placed into the Daisy^{II} Incubator, and after 30 min, 400 ml of strained ruminal fluid was added. Each feedstuff (in triplicate) was incubated in one jar (24 bags/jar), bags were removed at defined times of incubation, and handled as described for the IS bags. This procedure was repeated in order to obtain six replicates by feedstuff and incubation time.

All procedures related to ruminal fluid mixing and straining, preparation of the buffer solution, and manipulation at initiation and during incubations were performed under continuous flushing of CO_2. Additionally, the pH course of the ruminal/fluid buffer mixture during incubation was measured (OAKTON pH/mv/°Cmeter) at each jar and incubation time.

2.4. Chemical analysis

In all feedstuffs, dry matter and crude protein (CP) were determined (AOAC method no.: 984.13, 1990), and NDF, acid detergent fibre (ADF) and lignin were measured sequentially (Van Soest et al., 1991; without sodium sulfite in the neutral detergent solution) using an ANKOM^{200} Fiber Analyzer (ANKOM Technology Corp., Fairport, NY, USA). Neutral detergent fibre assayed with heat stable amylase (aNDF) was used for agro-industrial by-products and their residues from incubations. Neutral detergent fibre assayed without a heat stable amylase (NDF) was used for cultivated pastures and high dry matter forages and their residues from incubations. A heat stable α-amylase (ANKOM Alpha-Amylase) was included in the NDF solution used in the analysis of agro-industrial by-products and their residues from incubations. Neutral detergent insoluble N (NDIN) was determined by measuring N (AOAC method no.: 984.13, 1990) in the NDF residue. Fibre contents were expressed inclusive of residual ash. Hemicellulose (HEM) was estimated as the difference between NDF and ADF, while cellulose (CELL) was estimated as the difference between ADF and residue after digestion in 0.72:1 (v/v) sulfuric acid.

2.5. Calculations

To estimate degradation parameters (*in situ* and *in vitro*), data of DM and NDF disappearance at different incubation times were fitted to two models.

$$\text{Model 1 (Orskov and McDonald, 1979)}: \quad Y(t) = a + b\,(1 - e^{-kdt}), \quad t \geq 0$$

$$\text{Model 2 (Dhanoa, 1988)}: \quad Y(t) = a \quad \text{for} \quad t = L;$$
$$Y(t) = a + b\,(1 - e^{-kd(t-L)}), \quad \text{for} \quad t > L$$

where $Y(t)$ = fraction disappearance at time t, a (a_{DM}, a_{NDF}) = soluble or rapidly degradable fraction, b (b_{DM}, b_{NDF}) = insoluble but potentially degradable fraction, kd (kd_{DM}, kd_{NDF}) = degradation rate (h^{-1}), t = incubation time (h), and L = lag time (h). Degradation parameters were calculated using a non-linear procedure with Marquardt method (PROC NLIN) (SAS Institute Inc., Cary, NC), and parameters with the largest r^2, were reported. Effective degradability of DM (ED_{DM}) and NDF (ED_{NDF}) were calculated as $ED_{DM/NDF} = a + b \times kd/(kd + kp)$ when $L \leq 0$ (Orskov and McDonald, 1979), and as $ED_{DM/NDF} = a + (b \times kd) \times e^{-kd(L)}/(kd + kp)$ when $L > 0$ (Dhanoa, 1988). A constant ruminal passage rate (kp) of 0.06 h^{-1} was used.

2.6. Data analysis

The agreement between the two techniques was analyzed by the Bland-Altman method (Altman and Bland, 1983). Means of the paired measurements of disappearance (DM or NDF) obtained by the IS and IV Daisy^{II} in each incubation time (x-axis) were plotted against their difference (y-axis). The mean of differences between procedures, and the 95% limits of agreement of the mean of differences (2 standard deviations; more precisely 1.96) were calculated, and superimposed on the plot. The number of points that fell within the limits of agreement were then observed, and recorded. The Spearman and Pearson correlations (PROC CORR of SAS, 2000) were calculated; the former to test the relationship between the procedures differences and the size of measurements, and the latter to measure association between procedures. Correlations were considered significant at $P<0.05$.

The IS and IV data of disappearance were statistically analyzed according to the following models. The first model included feedstuffs, procedures, time of incubation and interactions (feedstuff × procedure, time × procedure and time × procedure × feedstuff), and dependent variables were IS and IV disappearances of DM and NDF at each incubation time. The second model (performed in order to evaluate the size of differences between procedures at 4 or 96 h) included time of incubation, and dependent variable was the ratio of disappearance of DM (and NDF) by the two procedures (IS/IV) for each feedstuff. The GLM procedure of SAS (SAS Institute, 2000) was used, and comparisons among means were carried out with the Tukey method; means were considered to differ when $P<0.05$.

Parameters of degradation of both procedures were compared by confidence intervals ($\alpha = 0.95$) because there were two replicates (two jars) in the IV Daisy^{II}. The standards errors used were obtained with the NLIN procedure of SAS (SAS Institute, 2000).

Table 1
Chemical composition of the feedstuffs evaluated (g/kg DM).

Feedstuff	DM[a]	CP	NDF	ADF	HEM	CEL	Lignin (sa)
White clover	144.4	311.8	305.3	213.0	92.3	151.3	61.7
Birdsfoot trefoil	169.2	306.6	386.8	220.3	166.5	127.6	92.7
Ryegrass	140.4	224.1	529.3	294.0	235.3	269.1	24.9
Oat	158.1	198.9	484.2	313.7	170.5	268.5	45.2
Alfalfa hay	968.9	176.1	593.1	357.3	235.8	274.2	83.1
Barley straw	963.4	27.9	824.2	538.4	285.8	459.8	78.6
Brewers' grains[b]	206.8	339.3	676.6[b]	232.5	444.1	172.3	60.2
Citrus pulp[b]	954.2	80.9	273.4[b]	190.0	83.4	161.8	28.2

[a] ADF: acid detergent fibre; CEL: cellulose; CP: crude protein; DM: dry matter (g/kg as fed); HEM: hemicellulose; Lignin (sa): sulfuric acid lignin. NDF: neutral detergent fibre.
[b] Values of aNDF: NDF assayed with a heat stable amylase heat stable α-amylase (ANKOM Alpha-Amylase) was included in the NDF solution.

3. Results

3.1. Chemical composition

As expected, variability in NDF cell wall content (NDF), and composition (CELL, HEM, and lignin (sa)) was observed among feedstuffs (Table 1). The NDF content ranged from 272 to 824 g/kg DM, and from to 127 to 460, 82 to 427, and 25 to 93 g/kg DM, for CELL, HEM, and lignin (sa), respectively. The NDIN of brewers' grains was 36 g/kg DM.

3.2. Comparison of in situ and in vitro disappearance

The plot of differences between mean values obtained by the IS and IV Daisy^{II} procedures (DIFISIV) for each feedstuff and incubation time (y-axis), against their mean (x-axis) for DM and NDF disappearance are presented in Figs. 1 and 2, respectively. No significant relationships were detected (Spearman $r = -0.047$ and -0.032; P=0.33 and 0.51 and $n = 64$ and $n = 62$ for DM and NDF disappearances, respectively) between DIFISIV, and the size of measurements for DM and NDF disappearance; therefore, the assumption of independence is not contradicted by the data. The Altman and Bland (1983) analysis indicated that the average of DIFISIV were 0.10 and 0.16, and the 95% limits of agreement between the two procedures ranged from 0.05 to 0.25, and −0.07 to 0.40 for DM and NDF, respectively. It was observed that 94 and 92% of DIFISIV in DM and NDF, respectively, were included between the 95% limits of agreement. However, most DIFISIV were positive, showing that systematically, disappearance values obtained by the IS were higher than IV Daisy^{II}. Therefore the IV Daisy^{II} underestimated disappearance values for both variables (up to 25 and 40% for DM and NDF, respectively), although the overall Pearson correlation between techniques were large ($r = 0.94$ and 0.88 for DM and NDF disappearance, respectively; P<0.05).

In the IV Daisy^{II}, at 0, 2, 4, 8, 24, 48, 72, and 96 h of incubation the pH averaged, respectively, 7.0 (±0.10), 6.7 (±0.11), 6.8 (±0.11), 6.7 (±0.10), 6.6 (±0.12), 6.46 (±0.23), 6.51 (±0.10) and 6.46 (±0.03).

In the comparison of DM (and NDF) disappearances by the IS and IV Daisy^{II} an interaction (P<0.01) between feedstuff, procedure and incubation time was detected. At early incubation times, some feedstuffs seemed to present similar DM or NDF disappearance with both procedures; while in others, disappearance was greater with IS (Table 2).

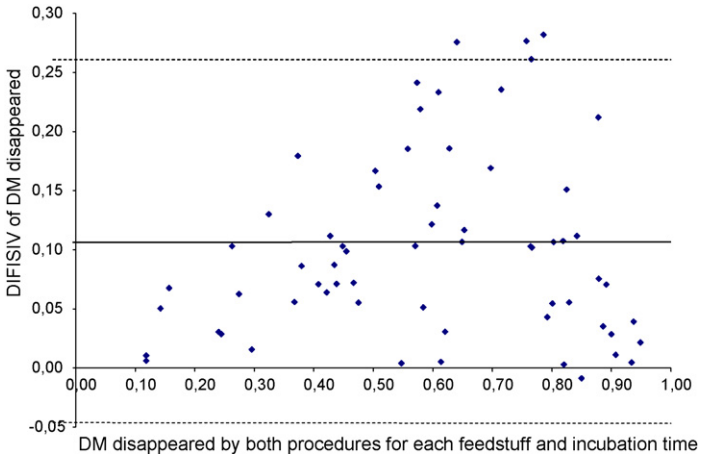

Fig. 1. Analysis of Altman and Bland (1983) for dry matter (DM) disappearance. Solid line correspond to the average of difference between mean values of DM disappearances obtained by the IS and IV Daisy^{II} procedures (DIFISIV). Dashed lines represent the limits of agreement, which are defined as the mean of differences plus and minus 1.96 times the standard deviation of the differences.

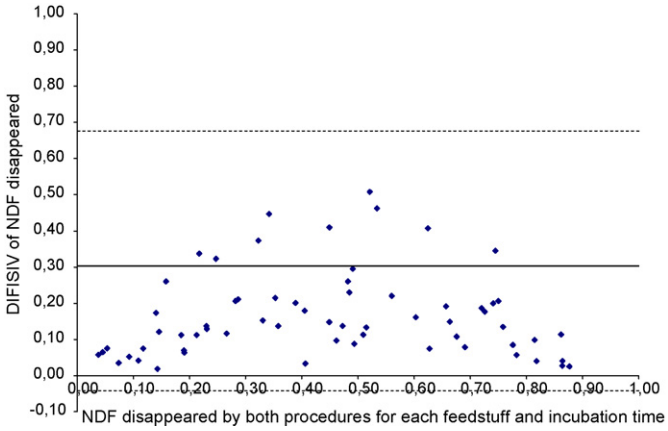

Fig. 2. Analysis of Altman and Bland (1983) for neutral detergent fibre (NDF) disappearance. Solid line correspond to the average of difference between mean values of NDF disappearances obtained by the IS and IV Daisy^{II} procedures (DIFISIV). Dashed lines represent the limits of agreement, which are defined as the mean of differences plus and minus 1.96 times the standard deviation of the differences).

Differences in the ratio IS/IV of DM disappearance at 4 and 96 h were less frequent than when FDN disappearances were considered. In ryegrass, alfalfa hay, barley straw, and brewers' grains, the DM disappearance ratio was similar at 4 and 96 h, while in the others feedstuffs, IS/IV seemed greater at 4 than at 96 h. However, in all feedstuffs the FDN ratio seemed greater at 4 than 96 h, excepting alfalfa hay where ratios were similar at 4 and 96 h.

3.3. DM and NDF degradability

The IS and IV Daisy^{II} disappearance data of DM and NDF fitted the model of Orskov and McDonald (1979), or Dhanoa (1988), with r^2 that exceeded 0.80. Differences between parameters (*a*, *b* or *kd*) estimated by IS and IV Daisy^{II}, were less frequent for DM than NDF, and when differences were detected the IS values were greater ($\alpha = 0.95$) than the IV Daisy^{II}. Main differences in DM degradation parameters estimated by IS and IV, were the size of potentially degradable fraction or its degradation rate; while, in NDF, main differences were registered in the soluble fraction, and the degradation rate of *b*. Additionally, in the IV Daisy^{II} lags times in NDF degradation were detected in birdsfoot trefoil and ryegrass, and a larger ($\alpha = 0.95$) one was observed in oat (Table 3).

Although no statistical analysis was conducted to compare EDs (Table 3), ED_{DM} calculated from parameters of degradation estimated from IS and IV Daisy^{II}, resulted in similar values only for alfalfa hay; meanwhile for the other feedstuffs, IS estimates were larger than IV Daisy^{II}. The ED_{NDF} estimated from the IS data, consistently gave higher values than IV Daisy^{II}.

4. Discussion

4.1. Chemical composition

Chemical composition of pastures, dried forages, and agro-industrial by-products evaluated were consistent with data reported by NRC (2000, 2001) and Preston (2009). An exception was the aNDF content of brewers' grains, which was higher than reported in previously cited publications. The high content of NDIN may explain differences (NRC, 2001); removing this fraction (as CP) from aNDF resulted in a value (452 g/kg DM) within the range reported elsewhere (NRC, 2001; Preston, 2009).

4.2. Comparison of in situ and in vitro disappearance

Altman and Bland (1983) analysis showed that IV Daisy^{II} underestimated DM and NDF disappearances compared to IS disappearances as reported by Ceballos et al. (2008). The larger IS/IV ratios at early than at late incubation times, agreed with Weiss (1994) and Ceballos et al. (2008) who conclude that maximum degradation may be achieved at the same end time point (96 h). The larger disappearance observed in IS than in IV Daisy^{II}, agreed with results reported elsewhere (Dewhurst et al., 1995; Varel and Kreikemeier, 1995) for IS—*in vitro* (Tilley and Terry, 1963) comparisons.

The larger disappearance obtained IS may respond to differences in pore size of *in vitro* and *in situ* bags, physical incubation conditions, and differences in microbial ability to degrade substrates in the early and late incubation times. Larger pore size in the IS than in the IV Daisy^{II} bags, and physical incubation conditions, such as pressure exerted on bag by rumen contractions during digestion and faster rates of rumen liquor flow through the bags (Lindberg and Knutsson, 1981; Ceballos et al., 2008; Dewhurst et al., 1995) in the IS procedure, could result in larger losses of particles and degraded compounds from the bags. Microbial ability to degrade substrates may be affected by multiple factors which could shock microbial inoculum

Table 2
Dry matter (DM) and neutral detergent fibre (NDF) disappearance by *in situ* and *in vitro* DaisyII procedures.

Feedstuff	Time (h)	DM disappeared[a]		NDF[b] disappeared[a]	
		IS	IV Daisy[II]	IS	IV Daisy[II]
White clover	4	0.73	0.49	0.51	0.14
	8	0.83	0.60	0.65	0.24
	24	0.92	0.84	0.81	0.68
	48	0.91	0.87	0.83	0.73
	72	0.91	0.89	0.81	0.75
	96	0.91	0.90	0.86	0.77
Birdsfoot trefoil	4	0.60	0.42	0.29	0.03
	8	0.69	0.50	0.41	0.09
	24	0.82	0.72	0.67	0.45
	48	0.83	0.78	0.67	0.60
	72	0.86	0.80	0.73	0.62
	96	0.82	0.82	0.73	0.65
Oat	4	0.46	0.39	0.17	0.08
	8	0.60	0.43	0.40	0.18
	24	0.82	0.71	0.74	0.54
	48	0.84	0.73	0.78	0.59
	72	0.87	0.76	0.81	0.64
	96	0.85	0.84	0.84	0.80
Ryegrass	4	0.51	0.42	0.25	0.12
	8	0.64	0.52	0.46	0.23
	24	0.81	0.77	0.72	0.58
	48	0.89	0.78	0.84	0.64
	72	0.90	0.75	0.85	0.65
	96	0.93	0.86	0.91	0.79
Alfalfa hay	4	0.26	0.29	0.15	0.10
	8	0.40	0.34	0.22	0.16
	24	0.55	0.55	0.42	0.39
	48	0.61	0.56	0.50	0.40
	72	0.64	0.60	0.54	0.44
	96	0.62	0.65	0.57	0.45
Barley straw	4	0.12	0.12	0.08	0.01
	8	0.17	0.12	0.11	0.06
	24	0.31	0.21	0.27	0.13
	48	0.42	0.34	0.41	0.25
	72	0.48	0.37	0.49	0.29
	96	0.50	0.44	0.52	0.37
Brewer's grains	4	0.31	0.24	0.27	0.16
	8	0.39	0.26	0.30	0.17
	24	0.50	0.40	0.43	0.29
	48	0.65	0.47	0.60	0.37
	72	0.67	0.55	0.61	0.47
	96	0.71	0.59	0.68	0.52
Citrus pulp	4	0.79	0.58	0.65	0.35
	8	0.90	0.63	0.83	0.39
	24	0.96	0.75	0.88	0.55
	48	0.96	0.94	0.87	0.85
	72	0.96	0.93	0.89	0.86
	96	0.94	0.93	0.89	0.85
Pooled SEM		0.011		0.014	
P procedure		<0.001		<0.001	
P feedstuff		<0.001		<0.001	
P procedure × feedstuff		<0.001		<0.001	

IS: *in situ*; IV Daisy[II]: *in vitro* Daisy[II]; SEM: standard error of the mean.
[a] DM disappeared and NDF disappeared are expressed as a proportion of unit.
[b] NDF disappeared values of brewer's grain and citrus pulp correspond to aNDF (NDF assayed with a heat stable amylase) disappeared values.

in *in vitro* procedures. Factors such as source of rumen inoculum, composition and nutrient availability of diets offered to animal donors, rumen sampling time, inoculum preparation, sustained anaerobic environment during inoculum preparation, composition of the buffer solution, relative proportions of inoculum and medium, and the pH during incubation, has been reported to bias *in vitro* data, particularly at early incubation times (Mould et al., 2005; Weiss, 1994; Mertens, 1993; Grant and Mertens, 1992).

Table 3
Dry matter (DM) and neutral detergent fibre (NDF) degradation parameters and effective degradability of dry matter DM and neutral detergent fibre NDF of feedstuffs obtained by *in situ* and *in vitro* Daisy[II] procedures.

Feedstuff	DM				NDF[a]			
	IS	IV Daisy[II]	$\alpha = 0.95$	SEM	IS	IV Daisy[II]	$\alpha = 0.95$	SEM
White clover								
a[b]	0.46	0.44		0.01	0.12	0	*	0.002
b[c]	0.45	0.46		0.01	0.71	0.8	*	0.011
kd[d]	0.27	0.05	*	0.01	0.16	0.04	*	0.006
ED[e]	0.83	0.65			0.64	0.32		
Birdsfoot trifolium								
a	0.41	0.41		0.01	0.05	0	*	0.003
b	0.43	0.41		0.01	0.66	0.66		0.001
kd	0.14	0.07	*	0.01	0.12	0.05	*	0.003
L[f]						6.4	*	0.003
ED	0.71	0.63			0.49	0.22		
Oat								
a	0.36	0.38		0.01	0.09	0.06		0.014
b	0.50	0.42	*	0.01	0.73	0.67		0.002
kd	0.08	0.08		0.01	0.09	0.05	*	0.004
L					2.5	3.8	*	0.004
ED	0.65	0.62			0.44	0.31		
Ryegrass								
a	0.38	0.37		0.01	0.14	0	*	0.004
b	0.52	0.41	*	0.01	0.74	0.76		
kd	0.08	0.09		0.01	0.07	0.06		0.001
L						1.2	*	0.003
ED	0.68	0.62			0.54	0.35		
Alfalfa hay								
a	0.16	0.17		0.004	0.05	0	*	0.002
b	0.47	0.43		0.007	0.51	0.44	*	0.008
kd	0.07	0.07		0.05	0.06	0.06		0.004
ED	0.41	0.40			0.31	0.22		
Barley straw								
a	0.08	0.09		0.003	0.06	0	*	0.001
b	0.46	0.50		0.007	0.51	0.49		0.03
kd	0.03	0.01		0.004	0.03	0.01	*	0.001
L					1.7	0		0.003
ED	0.23	0.18			0.21	0.07		
Brewer's grain								
a	0.16	0.20	*	0.005	0.07	0.05		0.003
b	0.52	0.46	*	0.013	0.56	0.52		0.019
kd	0.06	0.03		0.003	0.065	0.02	*	0.003
ED	0.43	0.35			0.36	0.18		
Citrus pulp								
a	0.39	0.39		0.007	0.23	0.18	*	0.007
b	0.56	0.55		0.007	0.65	0.69		0.009
kd	0.38	0.06	*	0.016	0.35	0.04	*	0.014
ED	0.87	0.67			0.78	0.46		

Fractions *a* and *b*, and ED are expressed as a proportion of unit; DM: dry matter; IS: *in situ* procedure; IV Daisy[II]: *in vitro* Daisy[II] procedure; NDF: neutral detergent fibre.

[a] NDF degradation parameters of brewer's grain and citrus pulp correspond to aNDF (NDF assayed with a heat stable amylase) degradation parameters.
[b] *a*: soluble fraction.
[c] *b*: insoluble but potentially DM/NDF degradable fraction.
[d] *kd*: degradation rate constant of DM/NDF (h^{-1}).
[e] ED: effective degradability of DM/NDF calculated assuming ruminal flow rate of 0.06 h^{-1}.
[f] *L*: lag time (h).

In this experiment, rumen sampling time, inoculum preparation, composition of the buffer solution, relative proportions of inoculum and medium, and pH could have influenced microbial activity. Rumen sampling time did not appear to be of importance because, 16 h elapsed between the last meal and liquor extraction, and it has been reported Mould et al. (2005) that, in animals fed more than once daily, rumen fluids sampled up to 20 h post-feeding present a stable microbial population. Less DM and NDF disappearance in the IV Daisy[II] in early incubation times, could be related to the time required for reestablishment of sufficient fibre-degrading microorganisms, because filtering rumen fluid may remove a large number of particles where fibre-degrading microorganisms normally attach (Varel and Kreikemeier, 1995). The composition of the buffer solution only included urea as source of N; the lack of amino N could have limited microbial action (Mould et al., 2005). The proportion of rumen fluid in the incubation medium used here, was 200 ml of rumen fluid/l incubation medium which was in the range (100–300 ml rumen fluid/l) reported (Mould et al., 2005) as adequate for microbial concentration in the incubation medium. Initiation of NDF degradation may be affected by low pH in the incubation media, resulting in lag times

when pH decreases from 6.8 to 6.0 (Grant and Mertens, 1992; Bossen et al., 2008). In this experiment, pH decreased from 6.7 (2 h) to 6.5 (96 h) indicating this factor would have not affected initiation of NDF degradation. The greater extent of digestion for the IS procedure at 96 h, may also be attributed to a sustained supply of nutrients to the microflora as animal consume feed. Conversely, no new dietary substrate was presented to the bacteria in the *in vitro* system, which could potentially limit the rate and extent of digestion due to limitation of specific nutrients (Van Soest, 1994).

Our results suggest that conditions related with inoculum preparation and N composition of the buffer solution, could have biased estimates of digestion kinetics in the IV Daisy^{II} procedure, resulting in a delay in the initiation of NDF degradation (lag times) in some feedstuffs.

4.3. DM and NDF degradability

The parameters *a*, *b*, and *kd* of DM and NDF estimated by IS were in reasonably agreement with the range of values reported for legumes (Hoffman et al., 1993; Marichal et al., 2010), grasses (Elizalde et al., 1992; Van Vuuren et al., 1992; Bargo et al., 2001; Barrios-Urdaneta et al., 2003), high dry matter forages (Barrios-Urdaneta et al., 2003; Varga and Hoover, 1983), and by-products (DePeters et al., 1997; Pereira and Gonzalez, 2004; Varga and Hoover, 1983). Lag times identified in NDF degradation of oat pasture and barley straw were as reported by Elizalde et al. (1992), Van Vuuren et al. (1993), Bargo (2002) and Barrios-Urdaneta et al. (2003). Exceptions were the high kd_{DM} and kd_{NDF} in white clover and citrus pulp, which could have resulted from their large (>0.66) DM or NDF disappearance in early hours (4 h) of incubation. Feedstuffs materials may differ greatly in brittleness, and therefore the distribution in size, and composition of particles passing a screen might vary considerably (Lindberg and Varikko, 1982). The early vegetative stage of white clover at harvest could result in a grinding profile including very small particles. A similar situation may have occurred in citrus pulp because dried pulps are excessively friable (Eisenhardt et al., 2009). This may result in large and fast losses of very small particles through the bag pores during the first hours of the incubation, which could overestimate kd_{DM} and kd_{NDF} (Orskov et al., 1980; Nocek, 1988).

Differences between procedures in a_{NDF} cannot be attributable to DM losses through bags pores, because in both procedures DM disappearance from bags was similar (P>0.05) at to; however, NDF concentration in bag residues was different. This suggested that differences between techniques could result from larger losses of fibrous particles in the IS procedure. In this procedure, ruminal motility probably put greater pressure in the bags than in IV Daisy^{II}, and the geometry of the fibrous particles associated with differences in shape of pores of bags used in both techniques, determined that fibrous particles left IS bags faster than IV Daisy^{II} bags (Van Soest, 1994). The slower kd_{DM} and kd_{NDF} estimated by the IV Daisy^{II}, and differences in lag times could be explained by the slower NDF disappearance observed in early fermentation hours in IV Daisy^{II}.

As result of differences between procedures in degradation parameters, ED_{DM} and ED_{NDF} values estimated by IV Daisy^{II} were lower than IS. However, when ranking feedstuffs according to their ED_{DM} estimated by both procedures, feedstuffs ranked equal; an exception was brewers' grains which ranked six in the IS but seven in the IV Daisy^{II}. Ranking similarities between procedures was associated with similarities in a_{DM} and b_{DM} estimates. An overall lack of agreement was established when ranking feedstuffs according to ED_{NDF} by both procedures, although feeds (oat, brewers' grains and citrus pulp) with similar a_{NDF} and b_{NDF} ranked equal. Summarizing, differences in ranking of feedstuffs could be associated to variations in values of $a_{DM/NDF}$ and b_{DMNDF} when estimated by both procedures because these values are the main determinants of ED.

5. Conclusions

IV Daisy^{II} underestimated DM and NDF disappearance values compared to IS, and the largest differences between procedures were observed at early incubations times. Both procedures ranked the fibrous feedstuffs similarly for ED_{DM}. This suggests that if one is primarily interested in comparing degradation potential of feedstuffs, the IV Daisy^{II} procedure appears to be a potentially useful tool; especially considering its advantages as a fast and simple technique.

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