



In vitro rumen feed degradability assessed with Daisy^{II} and batch culture: effect of sample size

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ABSTRACT – *In vitro* degradability with Daisy^{II} (D) equipment is commonly performed with 0.5g of feed sample into each filter bag. Literature reported that a reduction of the ratio of sample size to bag surface could facilitate the release of soluble or fine particulate. A reduction of sample size to 0.25 g could improve the correlation between the measurements provided by D and the conventional batch culture (BC). This hypothesis was screened by analysing the results of 2 trials. In trial 1, 7 feeds were incubated for 48h with rumen fluid (3 runs x 4 replications) both with D (0.5g/bag) and BC; the regressions between the mean values provided for the various feeds in each run by the 2 methods either for NDF (NDFd) and *in vitro* true DM (IVTDM) degradability, had R² of 0.75 and 0.92 and RSD of 10.9 and 4.8%, respectively. In trial 2, 4 feeds were incubated (2 runs x 8 replications) with D (0.25 g/bag) and BC; the corresponding regressions for NDFd and IVTDM showed R² of 0.94 and 0.98 and RSD of 3.0 and 1.3%, respectively. A sample size of 0.25 g improved the precision of the measurements obtained with D.

Key words: *In vitro* techniques, Rumen degradability, Feeds, Daisy^{II}.

Introduction - Daisy^{II} (D) is an equipment for analyzing DM and neutral detergent fiber (NDF) *in vitro* degradability. The technique entails digesting several feed samples in filter bags within glass jars which are rotated in insulated chamber. The amount of feed sample commonly introduced in the filter bag is 0.5g (Holden, 1999; Mabjeesh *et al.*, 2000), but others preferred 0.25 g (Robinson *et al.*, 1999; Spanghero *et al.*, 2003). Damiran *et al.* (2008) found significant differences of degradability due to sample size of 0.25 and 0.50 g/bag. It can be possible that a lower sample size could facilitate the release of soluble and fine particulate, while a larger sample size can exert a barrier effect, occluding the bags pores and limiting the rumen fluid passage. This work was aimed to evaluate which sample size (0.25 or 0.50 g/bag) allows to achieve a better correlation between the degradability values obtained with the D equipment and with a conventional batch culture technique (BC; Goering and van Soest, 1970).

Material and methods - The results of 2 previously conducted trials were analysed. In trial 1, 7 feeds (corn meal, soybean meal, dry sugar beet pulp, corn silage, alfalfa hay, grass hay and wheat straw), milled at 1mm, were simultaneously incubated (3 incubation runs x 4 replications) for 48h at 39°C both with D (0.50 g feed/filter bag) and BC (0.50 g feed/bottle), using the same rumen fluid collected from 3 donor cows and a buffer solution. Standard filter bags were used (F57; 4.3x4.8 cm; Ankom). Similarly, in trial 2, 4 feeds samples (concentrate mix; two different corn silages; alfalfa hay) were incubated (2 incubation runs x 8 replications) both with D (0.25 g feed/filter bag) and BC (0.50 g feed/bottle). At the end of each incubation, the residuals in the filter bags were analysed for NDF content with Ankom²²⁰ system while the residuals in the BC were filtered in gooch and analysed with a fibertech analyser (Goering and Van Soest, 1970). The *in vitro* true DM degradability was computed as $IVTDMD=100*[(DM_{feed}-NDF_{res})/DM_{feed}]$, where NDF_{res} was the residual NDF after incubation, and DM_{feed} was the amount of DM incubated. Data of each trial, either for NDFd and IVTDMD, were subjected to ANOVA using two models in which: i) the effect of technique was evaluated for each single feed; ii) the effect of technique was evaluated considering the various feeds as source of variation. The root of MSE (RMSE)

Table 1. LS means and variability parameters for NDF (NDFd, %NDF) and *in vitro* true DM degradability (IVTDMD, %DM) obtained with the Daisy¹ (D) and the batch culture (BC) techniques in trial 1 and 2.

Technique	NDFd			IVTDMD		
	Daisy	BC	RMSE ²	Daisy	BC	RMSE ²
	Mean ±SD ¹	Mean ±SD ¹		Mean ±SD ¹	Mean ±SD ¹	
Trial 1 (0.50 g/filter bag) ⁴ :						
Corn meal	63.5±12.3	81.4±4.5	11.3**	96.6±1.3	98.2±0.4	3.2**
Soybean meal	99.1±2.1	93.5±2.0	2.1*	100.0±0.6	99.1±0.3	0.6*
Dry sugar beet pulp	76.3±6.0	89.7±1.1	5.5**	87.9±3.3	94.7±0.5	3.1**
Corn silage	38.1±7.4	63.5±1.3	6.8**	73.0±3.5	84.0±0.6	3.2**
Alfalfa hay	48.1±3.3	51.7±2.6	3.2**	76.9±1.8	78.6±1.1	2.3**
Grass hay	46.3±3.6	61.9±2.9	3.4**	69.6±2.2	78.4±1.6	2.2**
Wheat straw	31.4±3.9	53.1±2.0	3.6**	46.4±3.4	63.4±1.6	3.2**
RMSE	6.4	2.6		2.6	1.2	
CV ³	11.1	3.7		3.3	1.0	
Trial 2 (0.25 g/filter bag) ⁵ :						
Concentrate mix	71.3±4.4	75.9±4.4	4.4**	92.9±1.1	94.1±1.1	1.1**
Corn silage 1	62.4±3.0	65.6±1.9	2.5**	80.6±1.5	82.3±1.3	1.4**
Corn silage 2	57.5±1.6	60.4±3.4	3.4**	74.6±1.0	76.4±2.0	1.6**
Alfalfa hay	42.1±2.3	44.0±4.0	3.7	73.5±1.1	74.4±1.7	1.4
RMSE	3.0	3.6		1.2	2.0	
CV ³	5.1	5.9		1.5	1.6	

Data within row for NDFd or IVTDMD significantly differed (** $P<0.01$; * $P<0.05$). ¹SD=standard deviation; ²RMSE=root of MSE; ³CV=coefficient of variation. ⁴Each value is a mean of 12 measurements (3 runs x 4 replications); ⁵Each value is a mean of 16 measurements (2 runs x 8 replications).

and the coefficient of variations (CV) were used as precision indexes. The mean values of degradability obtained with D equipment for each feed, in each trial and in each run, were compared by regression with the values obtained with BC (trial 1: 21 pairs of values; trial 2: 8 pairs of values).

Results and conclusions - In trial 1 (0.5 g feed/bag), the values of NDF degradability (NDFd) and IVTDMD obtained with D were significantly lower and less repeatable with respect to those obtained with BC (Table 1). The relationships for the NDFd and IVTDMD measurements provided by D (y) and BC (x) were: $y = -20.4 + 1.10x$ ($R^2 = 0.75$; RSD=10.9%) and $y = -27.7 + 1.25x$ ($R^2 = 0.92$; RSD=4.8%), respectively. The CV obtained with the D technique, both for NDFd and IVTDMD, were markedly higher than the corresponding CV achieved with BC.

With respect to trial 1, in trial 2 (0.25 g feed/filter bag), the degradability values obtained with the 2 techniques were much more similar both in term of mean values and CV (NDFd CV=5.14 and 5.85%, IVTDMD CV=1.46 and 1.59%, with D and BC, respectively). In trial 2 the relationships between the NDFd and IVTDMD measurements provided by D (y) and by BC (x) were: $y = 3.6 + 0.89x$ ($R^2 = 0.99$; RSD=3.0%) and $y = -1.15 + 0.99x$ ($R^2 = 0.98$; RSD=1.3%), respectively.

Result of this screening analysis indicated that the reduction of the sample size from 0.50 to 0.25 g of feed sample/bag (corresponding to 12 and 6 mg/cm² of bag surface) with the D allowed to achieve estimates of NDF and IVTDMD degradability more correlated to those provided by BC and less variable. This good agreement can be useful to exploit the advantage of each technique: D allows the simultaneous incubation of a large number of samples, giving benefit in term of labour and cost per determination, while BC gives the possibility of measuring not only the disappearance degree of substances but also the product of fermentations, such as volatile fatty acids and gas production.

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REFERENCES - **Damiran**, D., Del Curto, T., Bohnert, D.W., Findholt, S.L., 2008. Comparison of techniques and grinding size to estimate digestibility of forage based ruminant diets. *Anim. Feed Sci. Technol.* 141:15-35. **Goering**, H.H., Van Soest, P.J., 1970. Forage fiber analysis (apparatus, reagents, procedures and some applications). *Agr. Handbook No. 379*, USDA. **Holden**, L.A., 1999. Comparison of methods of *in vitro* dry matter digestibility for ten feeds. *J. Dairy Sci.* 82: 1791-1794. **Mabjeesh**, S.J., Cohen, M., Arieli, A., 2000. *In vitro* methods for measuring the dry matter digestibility of ruminant feedstuffs: comparison of methods and inoculum source. *J. Dairy Sci.* 83: 2289-2294. **Robinson**, P.H., Mathews, M.C., Fadel, J.G., 1999. Influence of storage time and temperature on *in vitro* digestion of neutral detergent fiber at 48h and comparison to 48h *in sacco* neutral detergent fiber digestion. *Anim. Feed Sci. Technol.* 80: 257-266. **Spanghero**, M., Boccalon, S., Gracco, L., Gruber, L., 2003. NDF degradability of hays measured *in situ* and *in vitro*. *Anim. Feed Sci. Technol.* 104: 201-208.