



COMMUNICATION

Estimating grass and grass silage degradation characteristics by *in situ* and *in vitro* gas production methods

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ABSTRACT

Fermentation characteristics of grass and grass silage at different maturities were studied using *in situ* and *in vitro* gas production methods. *In situ* data determined difference between grass and silage. Degradable fraction decreased as grass matured while the undegradable fraction increased. Rate of degradation (kd) was slower for silage than fresh grass. Gas production method (GP) data showed that fermentation of degradable fraction was different between stage of maturity in both grass and silage. Other data did not show any difference with the exception for the rate of GP of soluble and undegradable fraction. The *in situ* degradation characteristics were estimated from GP characteristics. The degradable and undegradable fractions could be estimated by multiple relationships. Using the three-phases model for gas production kd and fermentable organic matter could be estimated from the same parameters. The only *in situ* parameter that could not be estimated with GP parameters was the soluble fraction. The GP method and the three phases model provided to be an alternative to the *in situ* method for animal feed evaluations.

Key words: Grass, *In situ*, Gas production, Maturity

Introduction

Dynamic of nutrients digestion in the reticulo-rumen is one major determinant of utilization of feedstuffs by ruminants. Rate, extent, and synchronization of carbohydrates and protein degradation in feedstuffs can affect the rumen fermentation pattern, organic matter digestibility, and microbial protein synthesis and rumen health. Plant maturity and ensiling are some of the most important factors affecting the rate and extent of digestion of food components within the rumen and essential in predicting the nutritive value of grasses.

Incubation of feedstuffs at different time and a measure of *in situ* kinetic of rumen degradation (Mehrez and Ørskov, 1977) is used as basis in

many feed evaluation systems. Since the late 170s, measurement of in-vitro gas production (GP) has become increasingly popular for determining forage digestion characteristics and the kinetics of fermentation. Scientists are using classical gas production method described by Menke *et al.* (1979) and its modifications that are automated and use different electronics valves, pressure transducers and computers. The purpose of those investigations is the improvement of accuracy and the reduction of time for each sample, i.e. greater number of feed samples can be processed at any one time (Davies *et al.* 2000). The GP method is used as a research instrument to study fermentation characteristics of ruminant food, in breeding programs of grass and to search optimal degradable genotypes. Cone *et al.* (2002) showed a

Table 1. Fermentation characteristics of grass and silage samples, *in situ* method.

Sample	Maturity	S	D	U	k _d	FOM
grass	1	24,64	61,61	14,75	7,27	76,51
	2	25,65	59,69	15,66	7,27	76,05
	3	31,41	53,43	16,16	4,85	76,26
	4	23,63	48,28	29,09	6,06	65,27
	5	23,84	49,19	27,98	5,45	66,24
	6	21,92	46,46	32,62	4,85	62,32
silage	1	30,30	55,35	15,35	4,65	74,03
	2	34,44	51,31	15,25	4,34	74,55
	3	27,98	50,10	22,93	4,65	68,97
	4	36,06	42,42	22,52	3,74	70,33
	5	31,01	46,16	23,84	3,43	68,83
	6	28,08	48,38	24,54	3,03	68,63
	SEM	1,30	1,63	1,80	0,39	1,38
Sample significance		*	ns	ns	**	ns
Maturity significance		ns	**	**	ns	**

close relationship between *in situ* degradation characteristics and GP parameters for concentrate feedstuffs. The aim of this study is to investigate the possible relationship between *in situ* degradation characteristics of OM and GP characteristics in grass samples that differ in maturity and type of conservation (fresh and ensilaged).

Material and methods

The study was performed with grass *Lolium perenne* L. harvested at six stage of maturity. Grasses at different stage of maturity were harvested every seven days after first cut. All of the determination on samples, six grasses and six grass silages were performed two times in triplicate. Grass (approximately 45% DM) was ensiled for six week in 30 l laboratory silos. Rumen degradability of grass and silages was measured by the *in situ* procedure of Mehrez and Ørskov (1977). The soluble fraction (S) was determined by washing with cold tap water in a washing machine for 45 min. The residue after 336 h (14 days) incubation in the rumen fluid was considered undegradable fraction (U). The degradable fraction (D) was calculated as 100-S-U. Data was fitted to a first-order degradation model (Robinson *et al.*, 1986). Content of fermentable organic matter

(FOM) was calculated from *in situ* incubations. Determination of GP was carried out with a fully automated system as described by Cone *et al.* (1996). Curves of GP were fitted with a three-phase model, as described by Cone *et al.* (1996) and Groot *et al.* (1996). The non-linear model of the SAS package (SAS, 1989), was used to determine *in situ* and *in vitro* gas production parameters. Correlation between GP and *in situ* degradability parameters were obtained by the CORR procedure of SAS (1989).

Results and conclusions

In situ data (Table 1) for S ranged from 22% to 36% with no effect ($P \geq 0.05$) of grass maturity, but difference ($P \leq 0.05$) was determined between grass and silage. As grass matured D decreased ($P \leq 0.01$) while the U increased ($P \leq 0.01$) and ranged from 14.8% for the youngest plants to 32.6% for the oldest grasses ($r=0.86$). Rate of degradation (kd) decreased from fresh to ensiled ($P < 0.05$). Data of GP (Table 2) showed differences between stages of maturity for the fermentation of degradable fraction. Other data did not show difference ($P \geq 0.05$) with exception for rate of gas production. The *in situ* degradation characteristics were estimated from GP parameters. For multiple relationships,

Table 2. Fermentation characteristics of grass and silage samples gas production methods.

Sample	Maturity	a1	b1	c1	a2	b2	c2	a3	b3	c3
grass	1	77,77	3,13	0,90	125,24	7,37	2,75	52,52	33,33	6,16
	2	90,90	4,34	0,77	151,50	8,08	2,28	48,48	37,37	5,56
	3	104,03	2,63	1,05	117,16	8,89	2,44	38,38	28,28	4,95
	4	94,94	2,83	0,90	165,64	10,20	1,80	39,39	41,41	4,04
	5	98,98	2,02	0,84	160,59	11,01	1,78	41,41	40,40	5,25
	6	91,91	2,02	0,78	160,59	13,64	1,69	31,31	38,38	5,35
silage	1	80,80	4,85	1,25	109,08	9,29	2,89	51,51	32,32	5,86
	2	104,03	4,14	1,05	128,27	8,79	2,12	35,35	32,32	6,26
	3	108,07	3,43	0,78	144,43	10,91	2,31	44,44	38,38	5,86
	4	96,96	3,03	0,82	147,46	11,41	1,86	33,33	37,37	7,37
	5	101,00	3,54	0,80	143,42	13,43	2,08	50,50	40,40	4,65
	6	104,03	3,54	0,84	136,35	14,75	1,99	42,42	38,38	5,56
	SEM	2,73	0,25	0,04	5,20	0,68	0,11	2,08	1,17	0,25
Sample significance		ns	ns	ns	ns	ns	ns	ns	ns	ns
Maturity significance		ns	*	ns	*	***	**	ns	*	ns

a_n - is maximum gas production in ml; b_n - time at which half of the maximum gas production (a) is reached in h; c_n - parameter determine the shape of the curve.

independent variables were included in the model with ($P \leq 0.05$). The D and U fractions could only be estimated by multiple relationships ($R^2 = 0.77-0.86$). Using the three-phases model for GP, both kd and FOM could be estimated from the same parameters and the relationships were ($R^2 = 0.86-0.92$). Only S of *in situ* could not be estimated with GP parameters ($p \geq 0.05$).

Maturity of grass and silage caused a decreased degradability. The GP and the three phases model provided to be an alternative to the *in situ* method. *In situ* method is time consuming and impose a greater stress to animals than GP methods that need further investigations to become standard method for animal feed evaluations.

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