Use of *in vitro* gas production technique for predicting *in vivo* apparent digestibility and voluntary intake of feedstuffs for sheep

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Abstract

Twenty four Suffolk x Kent wether lambs were used to measure the dry matter (DM) digestibility and DM intake of twelve feeds, six being classified as "long forages" and the rest were milled "other forages". Animals and feeds were divided into three groups of 4x4 latin squares. A gas production assay was carried out using the Theodorou et al. (1994) technique and incubating the samples in either Theodorou (contains nitrogen) or Menke (contains no nitrogen) media. For gas production there were significative effects (P<0.001) of "media", "feeds" and "feed x medium", for both groups of forages. In vivo *DM digestibility and DM intake of "long forages" were highly correlated with* in vitro *DM digestibility of Menke medium (R^2 0.92 P<0.01, and 0.96 P< 0.001), but for Theodorou medium only digestibility was correlated (R^2 0.69 P<0.05). The* in vivo *digestibility of "other forages" was correlated with the Theodorou medium (R^2 0.77 P<0.05). Prediction of* in vivo *DM digestibility and DM intake of "long forages" was possible from Menke cumulative gas production (R^2 0.97 P<0.001 and 0.99 P<0.001) or Theodorou gas production (R^2 0.90 P<0.01 and 0.90 P<0.01). For "other forages" only DM digestibility was correlated (R^2 0.87 P<0.01) with the Theodorou medium and there was no correlation with the Menke medium.*

Keywords: *forages* in vivo *digestibility, intake,* in vitro *gas production*

Introduction

The quantity of feed consumed is fundamental to nutrition: it determines the level of nutrients ingested and therefore the animal's response and function. Digestibility and utilisation of nutrients are qualitative descriptions of the net feed intake (Van Soest, 1994). *In vivo* measurement of intake and digestibility may be a good guide to the potential performance of animals on particular diets, but their measurement is time consuming, involves a high labour input and requires a large number of animals of the same age, breed and sex to minimise experimental variation (Prasad *et al.* 1994). The prediction of digestibility and intake of feeds has been the subject of wide research, where several techniques have been proposed in an attempt to simulate the ruminant digestive process. The Tilley and Terry (1963) technique has been the most widely used *in vitro* technique, as has the enzymatic method of Jones and Hayward (1975). The *in situ* technique (∅rskov *et al.*1980), although inside the rumen, has the limitation of the restricted number of samples that can be measured at a given time. According to Menke *et al.* (1979), techniques such as the Tilley and Terry (1963), Jones and Hayward (1975), and ∅rskov *et al.* (1980) have made important contributions in feedstuff evaluation, but the techniques are still time consuming and imprecise. Looking for some other alternative method, Menke *et al.* (1979) developed a simple technique based on the Tilley and Terry (1963) method which consists of measuring gas production during *in vitro* incubation of feedstuffs in rumen liquor. Menke *et al.* (1979) achieved good results in predicting digestibility and metabolizable energy of feeds. Following the same procedure, Khazaal *et al.* (1993) had success in predicting voluntary intake and dry matter digestibility of legume hay. Khazaal *et al.* (1995) also predicted voluntary intake of graminaceous hay from the gas production technique. Similar results were obtained by Blummel and ∅rskov (1993) in predicting voluntary intake and dry matter digestibility of cereal straws.

Theodorou *et al.* (1994) proposed some changes to the Menke *et al.* (1979) technique. The changes involved the incubation of samples in glass bottles, the use of a pressure transducer for gas readings and the addition of nitrogen to the inoculum. Following the Theodorou *et al.* (1994) suggestions, Prasad *et al.* (1994) achieved good results in predicting digestibility of straws. Adesogan *et al.* (1995) and Sileshi *et al.* (1995) also investigated using the gas technique of Theodorou *et al.* (1994) to predict *in vivo* and *in situ* digestibility of forages.

The prediction of digestibility and intake through the gas production technique, has generated much research interest. Both the Menke *et al.* (1979) and the Theodorou *et al.* (1994) procedures have been researched, however, the two techniques have not been directly compared in any published paper. The present study involves assessing the reliability of the Theodorou and Menke media in the Theodorou *et al.* (1994) technique for predicting digestibility and intake of a variety of forages. Two contrasting groups of forages were compared i.e. "long forages" and "other forages". "Long forages" were hays, whereas "other forages" involved milled forages. It was hypothesised that the relationship between *in vivo* digestibility and intake would be different for the two groups of forages as also would the correlation between *in vivo* digestibility or intake and gas production.

Material and Methods

In vivo trial design

Twenty four Suffolk x Kent wether lambs, averaging 20 kg live weight, were placed in individual pens, with no bedding material. The lambs were divided into three groups of eight animals distributed in a 4 x 4 latin square. The diets were twelve commercial feeds (Table 1) which were divided into two groups, "long forages" and "other forages". "Other forages" were milled forages whereas "long forages" were unmilled hays. All the feeds were given as single diets *ad libitum* where four of them were randomly allocated in each latin square. Each latin square period consisted of three days of changeover, followed by fourteen days of adaptation to the new feed and the last seven days involved faecal collection for digestibility determination.

Chemical analysis and measurements

Representative samples for each feed in each period were taken and milled through a 1.0 mm sieve for chemical analysis and for the gas production assay. The DM content was determined by drying the samples to a constant weight at 65 °C. The concentration of nitrogen was determined by using the Khjeldal method and acid detergent fibre was determined according to the Goering and Van Soest (1970) method. At the end of each period, voluntary daily intake and apparent *in vivo* dry matter digestibility were determined

In vitro gas production

The method described by Theodorou *et al*. (1994) was used. This consisted of the anaerobic fermentation of dried ground feed samples with rumen microbes. One gramme samples (in triplicate) were transferred under anaerobic conditions into 125 ml capacity glass serum bottles together with 90 ml of a buffered microbiological medium, prepared according to Theodorou *et al.* (1994) or Menke *et al.* (1979) (Appendix A), and the bottles were sealed, with rubber stoppers. The mixture was inoculated with microbes prepared from fresh rumen liquor collected from two fistulated sheep fed hay and concentrate (75 : 25) and was fermented anaerobically at 39 °C.

After inoculating, all the bottles were readjusted to atmospheric pressure, using the pressure transducer. Bottles were then placed in the incubator and this time was considered as the starting point of the experiment i.e. 0^{h-1}. Gas production readings were made after 3, 6, 9, 12, 16, 20, 24, 28, 33, 39, 45, 52, 60 and 70 h of incubation. Measurement was made with a pressure transducer (Bailey and Mackey Ltd, Birmingham B42 1DE, UK) which measured the head space pressure in the bottles. At the end of the gas production run, the content of each bottle was vacuum filtered through pre-weighed filter crucibles (Sintaglass porosity 1, 70 ml capacity; Gallenkamp, Loughborough, UK) which were then oven dried overnight, cooled and weighed again.

Computation of data and statistical analysis

A spreadsheet Excel 5.0 PC program was used, where the cumulative gas production data were adjusted to that produced from 1g dry matter (DM) of sample fermented. *In vitro* digestibility (g/kg DM) was calculated assuming that all the residual DM after 70 h of fermentation was unfermented substrate. An analysis of variance, using the Statistical Analysis System (SAS 1985), was made to test the cumulative gas production at each time of reading, using feeds, medium (Theodorou and Menke media) and the interaction between feed x medium.

The cumulative gas production data were fitted to the France *et al.* (1993) model (MLP 1987) with the equation $y = A - B\,Q^{t} Z^{\sqrt{t}}$ where $Q = {}^{e-b}$, $Z = e^{-c}$ and $B = e^{bt + c\sqrt{t}}$. Here y denotes cumulative gas production (ml), t is incubation time (h), A is the asymptotic value for gas pool size (ml), t is the lag time and b(h^{-1}) and e ($h^{-0.5}$) are rate constants. A combined fractional rate (h^{-1}) of gas production (μ) was calculated as $\mu = b + c/2\sqrt{t}$, where t is the incubation time. A t-test was also made to evaluate the means of each parameter of the France *et al.* (1993) equation and for the *in vitro* DM digestibility of both media. The *in vivo* parameters DM digestibility and DM intake were correlated with cumulative gas production at each time of reading with *in vitro* DM digestibility, and with acid detergent fibre (ADF) and crude protein (CP) content of the two groups of feeds, using simple regressions.

Results

The *in vivo* DM digestibility, DM intake, and chemical analysis (CP and ADF) are presented in Table 1. The feeds were divided into two categories, being the "long forages" with high fibre and low protein content, whereas the "other forages" consisted of a variety of feeds. Hi fi light and grass pellets, although of high fibre content, were classified in the second group because of their small particle size making them more similar to the rest of "other forages".

Table 1

Table 2 presents the means of cumulative gas production at each time of reading, for both the "long" and "other" forages. Gas production was higher for the Theodorou medium than the Menke. For both groups of forages, the differences were highly significant throughout, except for the first 3 h. The "feed" factor was highly significant as expected. The "feed x medium" interaction of "long forages" was significant, in the late stage of fermentation, but the F values were small compared with those of the "other forages".

Table 2

Table 3 shows the parameters of the France *et al.* (1993) model for the Theodorou and Menke media. The means of the "long" and "other" forages were tested through a t-test to look at the differences between media for each component of the equation. There was a significant ($P<0.001$) difference for the rate of gas production (μ) for the "long forages", and also to a lesser extent ($P<0.01$) for the Z parameter of the same group. Other parameters were not significantly different. Table 3 also shows that DM digestibility values of both media were different ($P<0.001$).

Table 3

The relationships between *in vivo* DM digestibility and DM intake and cumulative gas production at each of the incubation times were investigated (Appendix C). Similarly the relationships between *in vivo* DM digestibility and DM intake and the other measured parameters were investigated (Tables 4, 5 and Appendix B). For "long forages", cumulative gas production was best correlated with DM digestibility at 3 h for Theodorou medium and at 28 h for Menke medium. For DM of "long forages" the best correlation with cumulative gas production occurred at 3 h for Theodorou medium and at 70 h for Menke, as is shown in Tables 4 and 5.

Table 4

Tables 4 and 5 also show that for "other forages" except for *in vivo* DM digestibility and cumulative gas production at 28 h for Theodorou medium (R^2 0.87 $P<0.01$) there were no significant correlations ($P>0.05$) between cumulative gas production and *in vivo* DM digestibility and DM intake.

Table 5

Discussion

In vivo DM digestibility and DM intake

The relationship between digestibility and intake was highly significant for the "long forages", but not for the "other forages" (Tables 4 and 5), thus confirming the hypothesis. In Table 1 it is seen that the two lowest DM digestibilities are from the grass pellets and the hi fi, where pellets had the highest intake, despite its high CP and ADF content. This is explained by Forbes, (1993) who commented "some feeds may be poorly digested, but pass through the digestive tract relatively quickly, thereby occupying space in the rumen for less time than a more digestible feed with a slower rate of passage such as the case of ground low quality forages. Although digestibility is relatively easy to measure, is probably not the most useful measurement for predicting intake".

A very different feature was observed in the case of sugar beet pulp which had the lowest intake rate of its group, but the highest DM digestibility; this could be explained by the fact of its highly soluble material content and highly digestible fibre. In this case was probably determined by the animal's metabolic regulation (Forbes, 1993).

Theodorou and Menke media

The differences between the two media in *in vitro* digestibility were large and highly significant (Table 2). As expected, there were also differences between feeds and their interactions. Both media were basically the same, with some exceptions. The Theodorou medium is more dilute and in the solution B (Appendix A) it has NH_4CO_3, whereas the Menke has none; NH_4CO_3 is a source of nitrogen and also a buffer. In solution B, Menke has four more grammes of $NaHCO_3$. In solution C, Menke has 3.75 g/l less Na_2HPO_4 than the Theodorou. Cysteine is a reducing agent in the Theodorou medium but is not used in Menke. The Theodorou medium contains nitrogen whereas the Menke does not. The nitrogen content is likely to be the main cause of differences between the two media. The Theodorou medium produced more gas and higher DM digestibility (Tables 2 and 3) especially in the case of "long forages" which generally had low CP content. According to Preston and Leng (1987), "deficiency of a nutrient needed by the rumen micro-organisms, will reduce the microbial activity and therefore reduce feed digestibility", particularly fibrous feeds, and probably the primary limitation to this is the supply of nitrogen to the micro-organisms in the bottles. However, in the present study, for "other forages" which had more CP content, the differences in *in vitro* digestibility between the two media, were less although the Theodorou medium gave a higher digestibility, this was not significant. ($P>0.05$) (Table 3).

There were also differences between the two media in cumulative gas production (Table 2) with the Theodorou medium producing more gas. The differences were probably due to presence of extra nitrogen and cysteine for microbial activity and causing it higher rates of fermentation and consequently production of gas (Russell and Trobel, 1993). This was particularly the case for "long forages". On the other hand, "other forages" were unlikely to have nitrogen deficiency (Table 1), even in the Menke medium, but it is possible that pH fell because the Menke medium had a lower buffering capacity, and consequently microbial activity and gas production may have been impaired. A question which arises is how much nitrogen has to be added to the Menke medium or how much nitrogen is in excess in the Theodorou medium?. A very recent investigation at the Natural Resources Institute, Chatham, by Rosales (1996) has addressed this question in the fermentation of tropical tree forages.

In vivo parameters and cumulative gas production

For "long forages" and the Menke medium, *in vivo* DM digestibility was highly correlated with gas production, reaching the highest correlation at 28 h of incubation. For the Theodorou medium the highest correlation occurred at 3 h, but was also high at 16 h (R^2 0.88), but after 16 h the correlation declined. "Long forages" DM intake was highly correlated with gas production with the Menke medium, being the highest correlation at 70 h (R^2 0.99), with the Theodorou medium the highest correlation being at 3 h (R^2 0.90) and thereafter declined (at 70 h; R^2 0.50) (Appendix C). These results show that DM digestibility and DM intake of "long forages" can be predicted at early stages of gas production using either Theodorou or Menke media, This confirms the results of Blummel and Ørskov (1993), Khazaal *et al.* (1995) and Prasad *et al.* (1994).

For "other forages", the gas production gave poor prediction of *in vivo* digestibility and intake, with the only good correlation (R^2 0.87) being between *in vivo* DM digestibility and the gas at 28 h for the Theodorou medium. Kibon and Ørskov (1993), using a Menke medium, also found a poor correlation between gas production and *in vivo* DM digestibility of browse species. In the present study with "other forages", the poor correlation between gas production and intake is understandable because of the fact that "other forages" were milled and intake would not be limited by physical capacity of the reticulo-rumen. The study by Kibon and Ørskov (1993) involved browse species; it is likely that the tannin content of the browse reduced the availability of nitrogen in the medium.

In the present study there were large and significant differences between gas production between the Theodorou and Menke media. For "long forages" the results suggest that *in vivo* DM digestibility and DM intake may be predicted from the *in vitro* gas production technique using either the Theodorou or Menke media. Furthermore, *in vivo* digestibility and intake of "long forages" can also be predicted from the *in vitro* digestibility of "long forages" using the Menke medium and digestibility from the Theodorou medium. The results are less clear for predicting *in vivo* digestibility and intake of milled "other forages" using the gas production technique. More research is required concerning milled forages.

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Table 1 *DM intake and in vivo DM digestibility of the feedstuffs used*

Feedstuffs	Crude protein (g/kgDM)	Acid detergent fibre(g/kg DM)	DM intake (g/kg $M.^{0.75}$ day)	s.d.	DM digestibility (g/kg)	s.d.
Long forages						
Dried rye grass	59	421	58	6.1	584	24
Timothy hay	95	409	55	7.2	561	39
Meadow hay	57	503	50	5.9	536	55
Rye grass hay	63	493	48	4.4	518	76
Straw 2	37	555	26	6.0	475	42
Straw 1	41	598	27	7.1	457	52
Other feeds						
Sugar beet pulp	99	258	53	23.3	817	65
Maize gluten feed	213	200	86	25.4	702	49
Wheat feed	171	170	58	10.2	672	51
Lucerne	192	367	120	9.4	636	24
Hi fi light†	108	515	67	22.1	570	58
Grass pellets	194	459	157	29.8	495	23

† A mixture of lucerne and oat straw

Table 2 *Cumulative gas production for different incubation times for Theodorou and Menke media; F value and significance of the effect of medium, feed and medium*feed interaction*

Time (h)	Cumulative gas production Theodorou (ml)	Cumulative gas production Menke (ml)	Medium F value	Feed F value	Feed*medium F value
Long forages					
3	14.6	12.5	3.3 NS	6.68 ***	0.19 NS
6	35.1	29.4	18.77 ***	61.82 ***	0.41 NS
9	53.0	43.0	43.57 ***	91.04 ***	0.52 NS
12	72.7	54.5	105.61 ***	90.25 ***	0.61 NS
16	99.4	65.9	273.54 ***	84.55 ***	1.02 NS
20	122.7	76.4	444.88 ***	84.41 ***	1.68 NS
24	142.3	85.5	557.84 ***	82.29 ***	2.46 NS
28	160.7	94.3	629.2 ***	77.61 ***	2.94 *
33	181.1	104.3	648.0 ***	67.65 ***	3.39 *
39	203.5	115.6	632.42 ***	54.08 ***	4.34 **
45	223.3	126.9	643.38 ***	49.01 ***	5.26 **
52	241.2	139.7	603.23 ***	44.42 ***	5.90 **
60	257.1	154.0	491.23 ***	38.41 ***	6.33 ***
70	270.2	168.4	369.49 ***	32.86 ***	6.34 ***
Other forages					
3	14.2	13.1	1.52 NS	0.52 NS	0.56 NS
6	46.3	36.1	40.33 ***	40.09 ***	8.6 ***
9	77.1	61.0	76.51 ***	97.93 ***	12.39 ***
12	104.2	83.4	90.56 ***	103.29 ***	14.42 ***
16	133.8	105.7	123.32 ***	89.92 ***	22.73 ***
20	158.8	123.9	176.54 ***	87.17 ***	31.75 ***
24	178.6	138.5	215.25 ***	81.66 ***	40.27 ***
28	197.0	151.6	258.90 ***	78.92 ***	52.13 ***
33	214.3	165.0	262.26 ***	65.98 ***	54.14 ***
39	230.1	178.5	233.41 ***	45.88 ***	47.95 ***
45	242.8	191.9	205.20 ***	35.30 ***	44.21 ***
52	255.0	205.5	194.31 ***	32.06 ***	42.81 ***
60	266.8	219.6	173.45 ***	31.96 ***	36.33 ***
70	277.0	233.6	140.48 ***	38.59 ***	25.85 ***

Table 3 *Estimated values for kinetics parameters from the fermentation and digestibility means of “l Menke media*

Feedstuffs	Lag time (T) (h)		Fractional rates Q (h^{-1})		Fractional rates Z ($h^{-0.5}$)		Gas pool size (A) (ml)	
	Th[1]	Mk[2]	Th[1]	Mk[2]	Th[1]	Mk[2]	Th[1]	Mk[2]
Long forages								
Dried rye grass	1.072	0.711	0.96757	0.99992	1.00737	0.98285	370.56	417.16
Timothy hay	0.92	1.273	0.96288	0.99651	1.03585	0.96149	284.4	497.51
Meadow hay	1.394	1.222	0.95982	0.99218	1.10171	0.96203	312.52	340.44
Rye grass hay	1.0	0.77	0.97192	0.99909	1.00532	0.97307	313.22	738.92
Straw 2	2.14	1.427	0.96499	0.98965	1.10149	0.96847	290.09	174.48
Straw 1	1.84	1.36	0.96366	0.99843	1.11448	0.96237	306.13	346.69
Mean	1.394[a]	1.127[a]	0.96514[a]	0.99596[a]	1.061[w]	0.968[x]	312.82[a]	446.42[a]
Other forages								
Sugar beet	1.01	1.71	0.94952	0.9995	1.02545	0.96304	379.07	886.28
Maize gluten	2.44	2.24	0.96838	0.95625	0.896	0.95323	287.64	274.33
Wheat feed	2.49	2.29	0.97646	0.94821	0.76876	0.92104	262.6	230.35
Lucerne	1.192	1.66	0.95048	0.97998	1.0746	0.98191	262.9	293.94
Hi fi light	1.295	1.02	0.96204	0.99675	1.09209	0.98418	321.3	749.33
Grass pellets	1.295	0.78	0.9528	0.95211	1.05527	1.0906	242.78	232.58
Mean	1.787[a]	1.616[a]	0.9599[a]	0.9721[a]	0.985[a]	0.982[a]	292.15[a]	447.47[a]

[1] Th = Theodorou medium
[2] Mk = Menke medium
[a a] = Non significant
[w x] = **
[y z] = ***

Table 4 *Relationship between in vivo DM digestibility and various measured parameters*

Parameter y	Parameter x	Equation
Long forages		
In vivo DMD (g/kg)	DM intake (g/kg)	y = 3.3655x + 374.19
	Acid detergent fibre (g/kg DM)	y = -0.6397x + 839.31
	Crude protein (g/kg DM)	y = 1.6795x + 423.18
	DM digestibility Theodorou (g/kg)	y = 0.5828x + 140.59
	DM digestibility Menke (g/kg)	y = 0.377x + 359.79
	Cumulative gas production at 3 h Theodorou (ml) [†]	y = 13.909x + 318.86
	Cumulative gas production at 28 h Menke (ml) [†]	y = 1.7417x + 357.41
Other forages		
In vivo DMD (g/kg)	DM intake (g/kg)	y = -1.9242x + 822.29
	Acid detergent fibre (g/kg DM)	y = -0.5765x + 837.88
	Crude protein (g/kg DM)	y = -0.7581x + 722.11
	DM digestibility Theodorou (g/kg)	y = 0.787x + 60.676
	DM digestibility Menke (g/kg)	y = 0.1263x + 567.14
	Cumulative gas production at 28 h Theodorou (ml) [†]	y = 2.4191x + 172.15
	Cumulative gas production at 70 h Menke (ml) [†]	y = 3.8487x - 250.52

[†] For data in Appendix C

Table 5 *Relationship between DM intake and various measured parameters*

Parameter y	Parameter x	Equation
Long forages		
Dry matter intake (g/kg)	*In vivo* DM digestibility (g/kg)	y = 0.2775x - 100.94
	Acid detergent fibre (g/kg DM)	y = -0.1766x + 131.54
	Crude protein (g/kg DM)	y = 0.5165x + 13.528
	DM digestibility Theodorou (g/kg)	y = 0.1534x - 56.521
	DM digestibility Menke (g/kg)	y = 0.111x - 3.8308
	Cumulative gas production at 3 h Theodorou (ml) [†]	y = 4.0294x - 14.932
	Cumulative gas production at 70 h Menke (ml) [†]	y = 0.2904x - 5.0544
Other forages		
DM intake (g/kg)	*In vivo* DM digestibility (g/kg)	y = -0.2609x + 259.49
	Acid detergent fibre (g/kg DM)	y = 0.1376x + 45.06
	Crude protein (g/kg DM)	y = 0.5362x + 291.97
	DM digestibility Theodorou (g/kg)	y = -0.2442x + 272.7
	DM digestibility Menke (g/kg DM)	y = -0.0478x + 121.11
	Cumulative gas production at 3 h Theodorou (ml) [†]	y =- 35.644x + 596.68
	Cumulative gas production at 6 h Menke (ml) [†]	y = -1.762x + 153.76

[†] For data in Appendix C

Appendix A Chemical composition of Theodorou and Menke media

Theodorou medium		**Menke medium**	
To give 900 ml of medium		To give 900 ml of medium	
Microminerals (A)	0.1 ml	Solution A	0.1 ml
Buffer (B)	200 ml	Solution B	200 ml
Macrominerals (C)	200 ml	Solution C	200 ml
Resarzurin	1.0 ml	Resarzurin	1.0 ml
Distillied water	500 ml	Distilled water	400 ml
Buffer (g/l)		Solution B (g/l)	
NH_4HCO_3			
NaCHO	35.0 g	Na HCO_3	39.0 g
Macrominerals (g/l)		Solution C (g/l)	
Na_2HPO_4 $12H_2O$	9.45 g	Na_2HPO_4 (anhydrous)	5.7 g
KH_2PO_4	6.2 g	KH_2PO_4	6.2 g
$MgSO_4$ $7H_2O$	0.6 g	$MgSO_4$ $7H_2O$	0.6 g
Reducing agent		Reducing agent	
Cysteine HCl. $1H_2O$	0.625 g		
Distilled water	95.0 ml	Distilled water	95.0 ml
1M NaOH	4.0 ml	1M NaOH	4.0 ml
Sodium sulphide	0.625 g	Sodium sulphide	0.625 ml

Appendix B *Correlation among all measured parameters*

Long forages

	in vivo DMD	DM intake	CG70 Th	CG 70 Mk	DMD Th	DMD Mk
in vivo DMD		0.93				
DM intake						
CG 70 Th	0.63	0.50				
CG 70 Mk	0.96	0.99				
DMD Th	0.69	0.58	0.98			
DMD Mk	0.92	0.96		0.95		
CP	0.49	0.57	0.01	0.55	0.04	0.57
ADF	0.92	0.85	0.38	0.86	0.47	0.90

Other forages

	in vivo DMD	DM intake	CG70 Th	CG 70 Mk	DMD Th	DMD Mk
in vivo DMD		0.5				
DM intake						
CG 70 Th	0.66	0.45				
CG 70 Mk	0.4	0.09				
DMD Th	0.77	0.54	0.72			
DMD Mk	0.02	0.02		0.05		
CP	0.1	0.39	0.56	0.02	0.17	0.24
ADF	0.54	0.22	0.06	0.11	0.37	0.36