

## PRELIMINARY OBSERVATION ON RELATIONSHIP OF DRY MATTER INTAKE BY SHEEP WITH FERMENTATION PARAMETERS, CHEMICAL COMPOSITION AND IN VIVO DIGESTIBILITY OF FORAGES

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### ABSTRACT

**T**he Pressure Transducer Technique (PTT) is based on measurement of gas production from fermentation of forages incubated with rumen inoculum buffered medium. Dry matter (DM) intake and in vivo digestibilities of six forage hays viz panicum, two cultivars of phalaris, Rhodes grass and natural pasture were determined using 36 (24 males and 12 females) local and cross bred (Menz x Awassi) Ethiopian highland sheep. Estimated parameters of gas production and in situ DM disappearances of the forages were determined and linear or multiple regression analyses were used to predict intake from estimated parameters obtained from PTT and Dacron bag methods and chemical compositions. The DM intake ( $\text{g}/\text{M}^{0.75} \cdot \text{d}$ ) ranged from 51.8 (Rhodes grass hay) to 60.8 (Phalaris hay1). The differences between the five forages was small ( $10 \text{ g}/\text{M}^{0.75} \cdot \text{d}$ ). The in vivo DM digestibility of the forages varied from 54.2 (Rhodes grass hay) to 65.9 (Phalaris hay2) g /100 g DM. DM intake was significantly related to neutral detergent fiber (NDF) content ( $P < 0.05$ ,  $R^2 = 0.62$ ), in vivo DM digestibility ( $P < 0.05$ ,  $R^2 = 0.66$ ) and the estimated parameters obtained from PTT ( $P < 0.01$ ,  $R^2 = 0.78$ ) and Dacron bag methods ( $P < 0.01$ ,  $R^2 = 0.78$ ). However, prediction of DM intake from the estimated parameters obtained from PTT and Dacron bag methods were more accurate ( $P < 0.01$ ) than prediction from NDF content. Only effective gas production (EGAS) obtained from PTT could be used in predicting DM intake ( $P < 0.01$ ). Of the estimated parameters obtained from Dacron bag method, rate of in situ DM disappearance was the most closely related to DM intake ( $P < 0.01$ ). DM intake was not related ( $P > 0.05$ ) with in situ rate and extent of DM disappearance parameters when used as factors in multiple linear regression. PTT can be used in ranking forages according to their ease of digestion and intake. However, the study used only six forages and the regression equations derived were based on data of low range of DM intake. More data are needed to assess whether PTT can be used in predicting intake of wide range of forages in Ethiopia.

### Introduction

While in vivo trials are accepted methods for determining the nutritive value of forages for ruminants, the high cost involved limit their use in forage quality evaluation. For this reason, various laboratory methods have been developed. The main indirect methods of forage

evaluation include fiber analysis (Goering and Van Soest 1970), two-stage in vitro digestibility of Tilley and Terry (1963), the Dacron bag rumen degradability method (Ørskov and McDonald 1979) and in vitro gas production method (Menke et al. 1979; Theodorou et al. 1991, 1994; Blümmel and Ørskov 1993; Khazaal et al. 1993). Intake of forages can be predicted from the rate and extent of their fermentation parameters. The Dacron bag method can be used to estimate both the rate and extent of forage DM disappearances in the rumen. However, the method is incapable of handling large number of samples at a given time and is therefore of limited value for routine use in a forage screening program.

The Pressure Transducer Technique (PTT) described by Theodorou et al. (1993) is based on measurement of gas production from fermentation of forages incubated with rumen inoculum buffered medium. Gas production during microbial fermentation is an indirect measure of forage degradability (Menke and Steingass 1988). The method is the quickest and the cheapest in estimating the fermentation pattern of gas production. The potential of PTT method as compared with the Dacron method for ranking fermentation profiles of four groups of Ethiopian forages was studied (Zinash 1994). The forages studied were three groups of improved forages harvested at different cutting intervals and a fourth group of crop residues and their botanical compositions. The results indicated that the estimated parameters from PTT ranked the forages similar to the Dacron bag method.

The accuracy of any indirect method of forage evaluation relies on the ability of the method to provide results that are correlated to animal responses such as intake and digestibility. Hence, the objective of this study was to relate estimated values of parameters of forages obtained from PTT and Dacron bag methods to their intake using six forages.

## Materials and Methods

### Experimental Forages and Preparation

The study was conducted at Holetta Research Center, IAR, Ethiopia. The forages used in the experiment were *Panicum* (*Panicum coloratum*) hay, hays from two cultivars of *Phalaris* (*Phalaris aquatica* "Sirroco"; *Phalaris aquatica* "Siroso"), Rhodes grass (*Chloris gayana* Massaba) hay, hay from grazing pasture of mixed species and oat (*Avena sativa*) hay. *Phalaris* hay was harvested at 50% flowering stage; *Panicum* and Rhodes grass hays at full heading and oat at milk stage. Native grass hay was harvested in November 1992 from the permanent pasture area, usually reserved for hay making. Hay from grazing composed of mixed species. The major species were *Pennisetum adones* (23.4%), *Andropogon abyssinicus* (12.3%), *Eleusine flocifolia* (11%), *Hyparrhenia rufa* (9.4), *Pennisetum schimperi* (10.9%), native clover (2.5%), and others (30.5%) (Alemu Tadesse, pers. comm). The forages were baled and stored under open-sided barn.

### Animals and Management

Thirty-six (24 males and 12 females) local and crossbred (Menz x Awassi) Ethiopian highland

sheep were stratified based on weight and sex, and allotted to the six forages at random. Four males and two females were assigned to each hay diet. The sheep were individually rationed 1.0 kg/day and fed twice daily at about 9.00 and 15.00 hr. During weighing the forage on offer, sub-sample was taken and pooled for a week for DM determinations. The sheep had free access to water and mineral block. The mineral block was purchased from the Third Livestock Project, Ministry of Agriculture (Ethiopia); each block weighing about 3 kg. Each block contained molasses 125 g, limestone 125 g, bone meal 1 kg,  $\text{CuSO}_4$  24 g,  $\text{MnSO}_4$  7.5 g,  $\text{ZnSO}_4$  20 g,  $\text{CoSO}_4$  0.25 mg and NaCl 1.25 kg (Third Livestock Project, pers. comm.). Daily forage on offer and refusal from each sheep was recorded for 30 days. Daily refusal from each sheep was removed at 7.00 hr and weighed. Daily refusal from each sheep was sub-sampled and bulked for one week for DM determination. The intake trial lasted for 22 days.

### In Vivo DM Digestibility

Total faeces were collected into canvas bags harnessed to four male sheep. Faeces collection was done at 7.00 hours before feeding the animals. Daily forage on offer and refusals were measured, and the refusals were sub-sampled from each sheep for DM determinations. Faecal samples, about 10% of the daily total collection from each sheep, were put in a fan draft oven (Gallenkamp, UK) on the same day and dried at 65 °C for 48 hr for DM determination. The digestibility trial lasted for eight days.

### In Vitro Gas Measurement Using PTT

Gas production from the six forages was determined using the PTT as described in Zinash (1994). The rumen fluid was withdrawn from six fistulated steers fitted with permanent rumen canulae. The incubation was done for 120 hr. At the end of the incubation period, the residue in each serum bottle was filtered using previously weighed Gooch crucibles (porosity 2) and dried over night at 65 °C. The volume of gas produced per g DM loss ( $Y$ , ml/g DM loss) was calculated by dividing the gas pool obtained from the equation by the weight DM loss (g DM loss/g DM incubated).

### In Situ Dm Disappearance Determination Using Dacron Bag Method

The nylon bags filled with the test forages were suspended in the rumen of fistulated steers for 6, 28, 48, 72, 96 and 120 hr. The diet of the steers consisted of grass hay offered ad libitum from native pasture and 3 kg of noug seed cake offered in two equal meals per day.

Each bag (inner size 7.5 x 10.5 cm; pore size = 50  $\mu$ ) was filled with 3 g of air dry sample and was suspended in the rumen just prior to the morning feeding (07.00 h). At the end of each incubation time, the bags were removed from the rumen, immediately rinsed with cold water

and washed by hand under running water until the effluent became clear. The clean washed bags were dried at 65 °C or for 48 hr, and weighed. Zero time washing DM loss was determined by washing the bags containing without rumen incubation. Duplicate bags were used for each forage sample.

## Chemical Analysis

Samples of the six forages were analysed for DM, OM, and N contents according to AOAC (1980) and fiber contents (NDF, ADF and permanganate lignin) according to the procedures described by Goering and Van Soest (1970).

## Statistical Analyses

The model proposed by France et al. (1993) was fitted to the exponential profile to estimate the rate and extent of gas production of the forages. In the model, rate of gas production is expressed by two fractional rates. A combined rate of gas production was calculated. The model by France et al. (1993) provides an estimate of effective gas production (EGAS) by combining all the parameters in a single equation. EGAS is an estimate of the amount of gas production from the forage degraded in the rumen and was calculated with an assumed ruminal passage rate.

In the Dacron bag method, the DM degradation constants were obtained after fitting the data to the exponential model described by Orskov and McDonald (1979). Effective DM disappearance (EDMD) of the forages was calculated with a passage rate of 0.02/hr.

Linear and multiple regression analyses were made on the relationships between DM intake and the estimated parameters obtained from PTT and Dacron bag methods and chemical compositions (SAS 1985).

## Results and Discussions

### DM Intake and In Vivo DM Digestibility

Chemical analyses, DM intake ( $\text{g}/\text{M}^{0.75} \cdot \text{d}$ ) and in vivo digestibility of the six forages are shown in Table 1. The DM intake ( $\text{g}/\text{M}^{0.75} \cdot \text{d}$ ) ranged from 51.8 (Rhodes grass hay) to 60.8 (Phalaris hay1). The in vivo DM digestibility of the forages varied from 54.2 (Rhodes grass hay) to 65.9 (Phalaris hay2) g DMD/100 g DM (Table 1).

Prediction of DM intake from chemical compositions and estimated parameters obtained from PTT and Dacron bag methods The mean gas production ( $\text{ml}/\text{g DM}$ ) at 24, 48 and 96 hr incubation time is shown in Table 2. Gas production from oat hay was higher than the gas production from the other forages.

Table 1. Chemical composition (g/100 g DM), DM intake (g/M<sup>0.75</sup>.d) and in vivo DM digestibility of the six forages

Forage	DM (g/100 g)	Chemical composition (g/100 g DM)					DM intake (g/M <sup>0.75</sup> .d)	In vivo DMD (g/100 g DM)
		OM	N	NDF	ADF	KmnO <sub>4</sub>		
<i>Panicum coloratum</i>	97	90	1.3	66	43	8	53.9 ± 0.616	54.8 ± 0.60
<i>Phalaris aquatica</i> "Sirroco"	97	91	1.5	60	40	4	60.7 ± 0.509	65.9 ± 0.61
<i>P. aquatica</i> "Siroso"	97	89	1.4	63	38	6	55.6 ± 0.584	61.0 ± 1.20
Rhodes grass hay	96	93	1.1	76	50	6	51.8 ± 0.696	54.2 ± 0.63
Hay from grazing pasture	96	93	1.5	71	43	8	53.9 ± 0.619	62.2 ± 0.75
Oat hay	96	92	1.4	74	44	8	54.2 ± 0.666	58.8 ± 0.6

Table 2. Gas production of forages (ml/g DM) at different incubation time

Forage	Gas production (ml/g DM <sup>1</sup> ) at different incubation-times		
	24 hr	48 hr	120 hr
Panicum hay	143 ± 3.0	207 ± 2.9	244 ± 1.8
Phalaris hay1	69 ± 2.8	247 ± 3.0	289 ± 2.0
Phalaris hay2	75 ± 2.1	259 ± 2.2	308 ± 2.0
Rhodes grass hay	149 ± 2.6	207 ± 2.3	250 ± 1.9
Oat hay	198 ± 2.4	269 ± 2.3	309 ± 1.9
Native hay from grazing pasture	161 ± 2.3	238 ± 2.5	288 ± 2.0

<sup>1</sup>Mean of four serum bottles

The estimated parameters of gas production and in situ DM disappearance of the hays are shown in tables 3 and 4, respectively. The rate of gas production (m) ranged from 0.032 to 0.048. Linear or multiple regression equations and R<sup>2</sup> derived from the equations in predicting DM intake from in vivo DMD, NDF content and estimated parameters obtained from PTT and Dacron bag methods are given in Table 5.

DM intake was significantly related to NDF content ( $P < 0.05$ ,  $R^2 = 0.62$ ), in vivo DM digestibility ( $P < 0.05$ ,  $R^2 = 0.66$ ) and the estimated parameters obtained from PTT ( $P < 0.05$ ,  $R^2 = 0.78$ ) and Dacron bag methods ( $P < 0.05$ ,  $R^2 = 0.78$ ). However, prediction of DM intake from the estimated parameters obtained from PTT and Dacron bag methods was more accurate ( $P < 0.05$ ), than prediction of intake from NDF content (Table 5).

Table 5 indicates that only EGAS obtained from PTT<sup>1</sup> could be used in predicting DM intake ( $P < 0.05$ ). The relationships between rate of gas production (m) and DM intake was not significant ( $P > 0.05$ ). Of the estimated parameters obtained from Dacron bag method, rate of in situ DM disappearance was the most closely related to DM intake ( $P < 0.05$ ). DM intake was not related ( $P > 0.05$ ) with in situ DM disappearance parameters of A, B and c, when used as factors in multiple linear regression.

Table 3. Estimated parameters of gas production and SE of six grass hays measured for intake and digestibility

Forages	Estimated and derived parameters						
	Q <sup>1</sup>	Z <sup>1</sup>	Gas pool <sup>1</sup> (ml)	Lag-time <sup>1</sup> (h)	Combined rate of gas production (m./h.) <sup>2</sup>	Y (ml/g DM loss) <sup>3</sup>	EGAS (ml/10 0 ml) <sup>4</sup>
Panicum hay	0.958 ± 0.0071	1.020 ± 0.048	246 ± 4.0	1.5 ± 0.40	0.043	379	43
Phalaris hay <sup>1</sup>	0.952 ± 0.0076	1.083 ± 0.0545	291 ± 3.7	1.2 ± 0.81	0.043	383	48
Phalaris hay <sup>2</sup>	0.956 ± 0.0080	1.060 ± 0.0640	311 ± 4.6	0.5 ± 0.82	0.043	428	46
Rhodes grass hay	0.972 ± 0.0071	0.941 ± 0.0535	255 ± 3.8	1.1 ± 0.63	0.037	396	42
Oat hay	0.965 ± 0.0072	0.945 ± 0.0552	311 ± 3.5	1.2 ± 0.90	0.043	475	45
Hay from grazing pasture	0.961 ± 0.0071	1.032 ± 0.0570	291 ± 3.5	0.5 ± 0.30	0.037	423	43

<sup>1</sup>The parameters were estimated from the equation of France et al. (1993).

<sup>2</sup>Combined rate of gas production (m) at 48 h incubation period was calculated as  $m \text{ (l/hr)} = c + (c_2 \times 20)$

<sup>3</sup>Y (ml/DM loss) = A fraction of the parameter/DM loss (g DM loss/100 g DM). <sup>4</sup>EGAS calculated with an assumed passage rate of 0.02/hr

Table 4. In situ DM disappearance from the nylon bags after incubation time and estimated parameters for six grass hays

Forages	In situ DM disappearance (g/100 g) by Incubation time (h)						Estimated parameters <sup>1</sup>					EDMD (g DM disappearance
	6	28	48	72	96	120	A	B	c	a+b	RSD	/100 g DM dis- appearance) <sup>2</sup>
Panicum hay	32	54	65	70	73	74	22	53	0.034	75	1.8	56
Phalaris hay <sup>1</sup>	40	63	72	76	78	80	29	50	0.041	79	1.2	63
Phalaris hay <sup>2</sup>	40	61	69	74	75	78	30	46	0.038	76		61
Rhodes grass hay	24	50	61	70	73	74	12	63	0.032	75	1.3	51
Oat hay	33	52	62	65	68	69	24	45	0.035	69	1.6	53
Hay from grazing pasture	32	58	68	74	76	78	21	57	0.037	78	1.3	58

<sup>1</sup>Parameters were estimated from the equation of Ørskov and McDonald (1979). A is the washing loss and B the insoluble but fermentable matter, B = (a + b) - A.

<sup>2</sup>EDMD was calculated with an assumed passage rate of 0.02/h.

There is evidence about the variability associated in intake of forages with fermentation pattern of forages (gas and DM disappearances) (Blümmel and Ørskov 1993, Khazaal et al. 1993). Other reports (Van Soest 1967 and Rohweder et al. 1978) showed that DM intake of forages can be predicted more accurately from NDF content. However, the relationship between intake and DM disappearance parameters or between intake and chemical composition are not consistent over the forages studied in the different references. Rohweder et al (1978) emphasised that the prediction of intake from NDF content varies between species and between forages grown at different locations. For example, for forages grown in temperate regions, the accuracy of predicting intake from NDF content is better than for forages obtained from tropical regions (Zinash 1994).

Table 5. Linear and multiple regression equations in predicting DM intake (g DM/M<sup>0.75</sup>.d) using estimated parameters from PTT and Dacron bag methods and chemical composition (N and NDF contents)

Parameter	Equation	R <sup>2</sup>	Level of significance
Rate of gas production (m <sub>g</sub> /hr)	28.0 (± 9.40) + 690.2(±239.16) m	0.5944	0.0448
EGAS (ml/100 ml)	-1.2 (± 13.03) + 1.3(± 0.29)EGAS	0.7793	0.0125
Rate of in situ DM disappearance (hr) (c)	24.1(± 7.70) + 852.1(± 211.58)DM	0.7527	0.0158
EDMD (g DM disappearance/100 g DM disappearance)	23.7(± 9.75) + 0.5(± 0.17)EDMD	0.6524	0.0322
In situ DM parameters of A, B, c	24.1(± 20.50) -0.1A(± 0.86) -0.1(± 0.50)B + 10008.0(± 1036.10) c	0.5124	0.2778
In vivo DMD (g/100 g DM)	20.5(± 10.53) + 0.6(± 0.18) DMD	0.6604	0.0307
NDF content (g/100 g DM)	83.2 ± (9.39) - 0.4(± 0.14) NDF	0.6180	0.0394
N content (g/100 g)	36.5(± 10.28) + 13.5(± 7.48) N	0.3119	0.1450

There were differences in digestibility of the forages at the same level of intake (Table 1). This could be responsible for low prediction of intake from DM digestibility of the forages. Other reports have also shown the same results (Milford and Minson 1966, Hovell et al. 1986 and Khazaal et al. 1993). Milford and Minson (1966) concluded that digestibility is not always a good predictor of intake of tropical grasses. This indicated that factors other than digestibility per se involved in diet voluntary intake of some of the forages.

From the data presented in literatures (Ørskov et al. 1988, Von Keyserlingk and Mathison 1989, Carro et al. 1991, Nandra et al. 1993, Khazaal et al. 1993, Blümmel and Ørskov 1993, Kibon and Ørskov 1993), regression analyses on the relationships between intake and estimated parameters obtained by PTT (EGAS) and Dacron bag method (EDMD) and chemical composition (N, NDF) were made. Based on the regression coefficient (R<sup>2</sup>) obtained from the regression equation as well as the level of significance, prediction of intake using Dacron bag method was not consistent across the cited references. In agreement with the present results, Carro et al (1991) found that from the estimated parameters of DM disappearances, rate of in situ DM disappearance was the parameter from which the intake of the forages can be predicted. Other reports, however, showed significant relationships between potential DM disappearance (a + b) (Hovell et al. 1986) or in situ DM disappearance fitted of constants (Ørskov et al. 1988 and Khazaal et al 1993). It was suggested (Hovell et al. 1986, and Carro et al. (1991) that further research is required to examine whether different parameter are needed to predict intake of different forages.

Only few reports were available on prediction of intake of forages from their estimated parameters of gas production (Blümmel and Ørskov 1993, and Khazaal et al. 1993) or volume of gas production of forages after short incubation time (24 to 48 hr) (Kibon and Ørskov 1993). The EGAS calculated from the data of Blümmel and Ørskov (1993) and Khazaal et al. (1993) was significantly (P < 0.01) related to intake.

The relationships between EGAS and DM intake presented in this study are in agreement with other results (Blümmel and Ørskov 1993, and Khazaal et al. 1993). Blümmel and Ørskov

(1993) and Khazaal et al. (1993) did not use the parameter EGAS in predicting intake, but the data reported in these references were used to analyse the relationship between DM intake and EGAS (Zinash 1994). Kibon and Ørskov (1993) studied the relationship between intake and gas production and they reported that volume of gas production recorded between 24 to 48 hr explained 87% of the variation in intake of browse trees.

## Conclusions

The present study only used six types of forages and the regression equations derived were based on data of low range of DM intake. However, bearing in the limited range of data, the results indicated the potential of PTT in predicting intake of forages. In addition, PTT can also be used in estimating *in vivo* DM digestibility of the forages 90% of the variation in *in vivo* DM digestibility of the six forages was explained by EGAS. PTT, therefore, has a distinct advantage over two-stage *in vitro* Tilley and Terry 1963 in that it is capable of predicting both intake and digestibility.

EGAS might be expected to rank the forages according to their ease of digestion and intake. More data are, however, needed to confirm whether EGAS can be used in predicting intake of wide range of forages in Ethiopia.

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