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A Practical Gas Production Technique to Determine the Nutritive Value of Forages: The UC Davis Approach

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INTRODUCTION

Forages can be chemically assayed for a number of constituents that define their nutritive value. However the energy content of a forage, which is the nutritive characteristic that often defines much of its economic value, is not a chemical constituent and cannot be chemically assayed. This situation has challenged agronomists and ruminant nutritionists for decades, and provided the impetus for development of numerous equations and systems that purport to estimate the energy value of forages from one or more chemical constituent(s). These equations and systems may have worked well, or not so well, although it is virtually impossible to critically evaluate them, since energy values of forages have not been regularly measured in ruminants since the late 1960's in North America. Thus there are no 'standards' to which predicted energy values can be critically compared.

It has long been recognized that the two key factors that determine the energy value of a forage are its content of fat, due to its high energy value, and the digestibility of its structural fiber (i.e., NDF), due to its high content in forages by definition. The former can be dealt with by chemical analysis, and is of minor importance in many forages, although the latter has proven to be more difficult to estimate.

In North America, the tendency has been to rely upon the basic similarity of structural fiber, within a forage type, to develop unique energy prediction equations for each forage type. This approach has been followed by the National Forage Testing Association (NFTA), which lists numerous equations at its web site to predict the total digestible nutrient (TDN) value of specific forages. A problem with this approach is that often the botanical description of the forages must be known in order to decide which equation to use. This provides intractable problems for unknown and mixed forages. In addition, these equations tend to be region specific. This can be a problem for forages, such as alfalfa hay, that are transported to markets outside their region of origin and for commercial laboratories that receive forages from all over North America and, in some cases, the world. In contrast, European countries have tended towards the use of *in vitro*

digestibility procedures to estimate actual fiber digestibility. This approach eliminates concerns about accurate botanical description of the forage, but introduces the complexity, cost and uncertainty of *in vitro* procedures.

In vitro procedures have developed over the years and now fall into two basic types. The 'traditional' procedure incubates small amounts of the test forage with rumen fluid for a defined time period. The incubation is terminated, and the residual dry matter (DM) and/or NDF is determined gravimetrically. This destructive procedure has clear limitations if more than one incubation time point is of interest, for example to create a rate of digestion. Thus its commercial use is generally restricted, due to cost, to a single time of incubation to create a digestible NDF (dNDF) proportion (Quaife, 2002). Needless to say, agreement on the most appropriate time of incubation has eluded agreement and incubation times between 30 and 72 h are utilized in practice. An alternate *in vitro* procedure, widely used in Europe, also incubates small amounts of the test forage with rumen fluid. However in this procedure the amount of gas produced in the fermentation is cumulatively collected and recorded continuously with automated equipment or recorded at defined time intervals manually. The advantage of this procedure is that the incubation need not be terminated to measure the extent of digestion, although the disadvantage is that the amount of gas collected is only an indicator of the amount of carbohydrate fermented, rather than a measurement per se. Thus the amount of gas produced must be related to the energy content of the forage.

The purpose of this article is to discuss the use of a gas production procedure utilized at UC Davis to estimate the energy value and intake potential of forages.

THE UC DAVIS GAS PROCEDURE

Incubations are performed using 30 ml of buffered rumen fluid according to Menke and Steingass (1988). Approximately 200 mg of feed is weighed and placed into a 100 ml graduated glass syringe. Pistons are lubricated with Vaseline and inserted into the syringes. Buffer and mineral solution are prepared and placed in a water bath at 39^{o}C under continuous flushing with CO_2. Rumen fluid is collected from cows or sheep fed a high forage diet into a pre-warmed thermos flask. The rumen fluid is filtered and flushed with CO_2, and the mixed and CO_2 flushed rumen fluid is added to the buffered mineral solution (1:2 v/v), which is maintained in a water bath at 39^{o}C, and combined. Buffered rumen fluid (30 ml) is pipetted into each syringe, containing the feed samples, and the syringes are immediately placed into the water bath at 39^{o}C (Blummel and Ørskov, 1993). Three syringes with only buffered rumen fluid are incubated and considered as the blank. The syringes are gently shaken every 2 h, and the incubation terminated after recording the 72 h gas volume. Total gas values are corrected for the blank incubation, and reported gas values are expressed per g of DM.

This gas method has been standardized and validated as a method to create an energy prediction equation using data from 400 digestibility trials (*in vivo*) and the corresponding *in vitro* gas production tests (Menke and Steingass 1988).

INTERPRETATION OF THE GAS DATA

Gas produced by fermentation arises largely from the carbohydrate fraction of the forage, since ash does not ferment, fat produces no gas, and protein produces very little gas. If the amount of gas is recorded frequently, then a gas generation curve can be created (Figure 1), which detects differences both among forages in rate and extent of digestion of carbohydrates. While a single rate constant will generally fit these curves, total gas production is actually the sum of two underlying curves that reflect the rapid fermentation of NSC and the slower fermentation of NDF (Figure 2). While it is theoretically possible to separate these curves mathematically, the repeatability of description of the underlying curves will be poor. Although some current ration evaluation software requires rate constants for these fractions, their true interpretative value is obscure. The approach utilized by our group, as well as some groups in Europe, is to select specific time points to record the amount of gas produced and use these values to quantitatively predict the energy value of the forage, as well as qualitatively predict the impact on intake potential.

Energy Estimation: The energy value of a forage can be calculated from the amount of gas produced at 24 h of incubation with supplementary analyses of crude protein (CP) and crude fat (CF). This approach was developed by the research group in Hohenheim (Germany) and is based upon extensive *in vitro* incubation of forages that had their actual energy value determined in ruminants (Menke et al. 1988). The original equation calculated the energy value as ME (metabolizable energy) in MJ/kg, although we have modified the equation and converted it to NE_l (Mcal/lb) for commercial use. The equation utilized is:

$$NE_l \text{ (Mcal/lb)} = (2.20 + (.0272*\text{Gas}) + (.057*\text{CP}) + (.149*\text{CF}))/14.64$$

Where: Gas is 24 h net gas production (ml/g DM)
CP is crude protein (% of DM)
CF is crude fat (% of DM)

The main advantage of this approach is that the equation is applicable to any forage from any geographic or agronomic area. Thus there is no need to assess incoming samples to determine the most appropriate energy equation to apply. The secondary advantage is that the calculated energy value of the forage reflects its chemical components as well as the rate at which the carbohydrates ferment in the rumen. A disadvantage of the approach is that a relatively time consuming (24 h) 'wet' procedure is required and access to rumen fluid is necessary. The former has been considered a difficulty due to variability, although repeatability in our laboratory is very high. The latter has also been considered to be a primary limitation of *in vitro* procedures. However we (Robinson et al. 1999) have shown that delays of up to 6.5 h between collection of rumen fluid and initiation of the incubation had no effect on digestibility of NDF. Most commercial laboratories in the USA are within 6.5 h of cows.

Voluntary Intake: In general, the energy value of forages is positively correlated to voluntary intake. This is because the energy value of most forages is heavily influenced by the level and fermentability of NDF. Typically, as the level of NDF in a species of forage increases, its intake potential declines. This is because, within most forage species, there is a strong negative correlation between NDF and digestible NDF (i.e., dNDF). Thus NDF level is a strong predictor of voluntary intake within a species of forage. However NDF, among species, is not the same chemically or in its intrinsic resistance to microbial degradation in the rumen (i.e., there is no correlation between NDF and dNDF among forage species). For example, NDF of grasses is generally more resistant to microbial degradation than NDF in legumes (i.e., grass NDF has a lower dNDF proportion than legume NDF). Thus NDF alone is a poor predictor of voluntary intake among species of forage.

But there are exceptions. Secondary compounds such as nitrates, saponins, tannins and oxylates, which are more common in tropical than temperate forages, can cause voluntary intake of any particular forage to be lower than that predicted based upon its level of NDF and its proportion of dNDF. Thus while secondary compounds can suppress voluntary intake, they will not elevate it as the NDF/dNDF levels of a forage fix that upper bound or, in a sense, maximum potential voluntary intake.

The gas production approach offers the possibility to generate data that can provide an insight into the ways that cattle will respond to a forage. Because the gas production technique records the amount of gas produced at various time intervals, the procedure can be used to create a gas generation curve over time, from a single incubation, that is the sum of gas produced from the NSC and the NDF. While it is theoretically possible to mathematically separate these curves, the repeatability of description of the underlying curves is poor. Thus a practical solution is to determine the amount of gas produced at specific times and utilize that to assess forage quality. In the UC Davis approach, gas production is recorded at three times of incubation (i.e., 6, 24 and 72 h) and these values are interpreted relative to the carbohydrate fractions. Thus gas produced up to 6 h of incubation is considered to estimate the extent of fermentation of NSC, while gas produced between 6 and 24 h is considered to estimate the amount of NDF that will be digested in cattle at high production levels. Finally, gas produced between 6 and 72 h of fermentation is considered to estimate the amount of NDF that will be digested in cattle fed at maintenance intake levels. Gas produced from several samples of several California forages are in Table 1.

The quantities of gas generated at 6, 24 and 72 h of incubation can be used to qualitatively assess intake potential of forages both among and within forage species. They can also be used to assess the extent of short term (through 6 h) fermentation, which may be very important in determining the intake potential of mixed rations, of which forages comprise only a portion. Indeed while the NDF and dNDF levels of forages govern their maximum voluntary intake, most cattle are fed mixed rations in which NDF levels are relatively low and will, in many cases, not govern maximum voluntary intake of the mixed ration. For example, in many commercial dairy rations total levels of NSC can approach, or even exceed, 40% of total DM making it quantitatively more important

than NDF. In such cases, it is the rapid fermentation of NSC leading to high levels of volatile fatty acids (VFA), and lactic acid in some cases, that depresses rumen pH and suppresses voluntary intake of the mixed ration. In cases where the NSC level of the diet is high, a rapid fermentation of it, measured by 6 h gas generation, will be negative to DM intake by allowing rumen microbes to create VFA and lactic acid and ammonia at a much faster rate than it can be absorbed, or utilized by other microbes, thereby suppressing intake. Conversely if levels of NSC are low, higher 6 h gas production will be positive to DM intake by providing rumen microbes with the nutrients that they require to grow and ferment fiber, thereby stimulating intake.

We believe that gas production of individual forages are valuable predictors of their voluntary intake potential when fed alone or in mixed rations.

CONCLUSIONS

Forages are grown as feeds for cattle and terms used to describe its value must have a basis in animal biology. Classifications of forages using relative terms are obsolete. The most appropriate quantitative descriptor of forage quality is its energy value, which in North America is net energy of lactation (NE_l). The most appropriate qualitative descriptors of forage quality are those that describe its impact on voluntary intake of mixed rations, in which forages comprise only a portion.

In most forages grown in temperate areas the positive overall relationship between the energy value of a forage and its intake potential makes measurement of other forage characteristics moot. However as commercial cattle produce higher levels of products, and eat higher amounts of DM to support that production, the importance of forage characteristics that impact intake of mixed rations, of which they comprise only a portion, becomes of paramount importance. The gas production procedure is a relatively inexpensive method that offers the ability to quantitatively determine the NE_l value of forages, as well as provide indicators of voluntary intake of the forages themselves, as well as the mixed rations to which they are incorporated.

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Table 1. *In vitro* gas production of some samples of California forages

		at 6 h	at 24 h	6-24 h	at 72 h	6-72 h	rate
		----------	ml of gas/g DM		----------		%/h
Alfalfa Hay		107.1	179.9	72.8	198.4	91.3	13.08
		109.2	207.3	98.1	222.3	113.1	11.29
		111.6	211.2	99.6	226.0	114.4	11.34
	Mean	**109.3**	**199.4**	**90.2**	**215.6**	**106.3**	**11.90**
Alfalfa Silage		70.1	137.4	67.3	162.1	92.0	10.26
		96.6	195.9	99.3	216.1	119.5	9.88
		132.3	203.6	71.4	207.2	75.0	16.91
	Mean	**99.7**	**179.0**	**79.3**	**195.1**	**95.5**	**12.35**
Bermuda Grass		52.0	187.2	135.2	231.4	179.4	6.12
Corn Silage		71.1	201.6	130.5	268.7	197.6	5.64
		74.3	193.5	119.2	251.2	176.9	6.31
		76.6	241.4	164.8	309.1	232.5	5.70
		77.2	217.5	140.3	275.4	198.2	6.09
		78.0	204.0	126.0	262.5	184.5	6.34
		83.3	242.3	159.0	305.1	221.8	6.14
		84.6	252.4	167.8	302.3	217.7	6.69
		88.8	220.8	132.0	284.3	195.5	6.24
		92.9	220.3	127.4	270.8	177.9	7.01
		105.5	261.1	155.6	334.8	229.3	6.31
		126.2	257.9	131.7	313.6	187.4	8.75
	Mean	**87.1**	**228.4**	**141.3**	**288.9**	**201.8**	**6.47**
Sudan Grass		65.3	170.5	105.2	233.4	168.1	5.48
Wheat Silage		82.5	191.2	108.7	230.3	147.7	7.39
		96.9	219.0	122.1	258.4	161.5	7.84
	Mean	**89.7**	**205.1**	**115.4**	**244.3**	**154.6**	**7.62**

Figure 1. Gas production profiles of samples of samples of three forages.

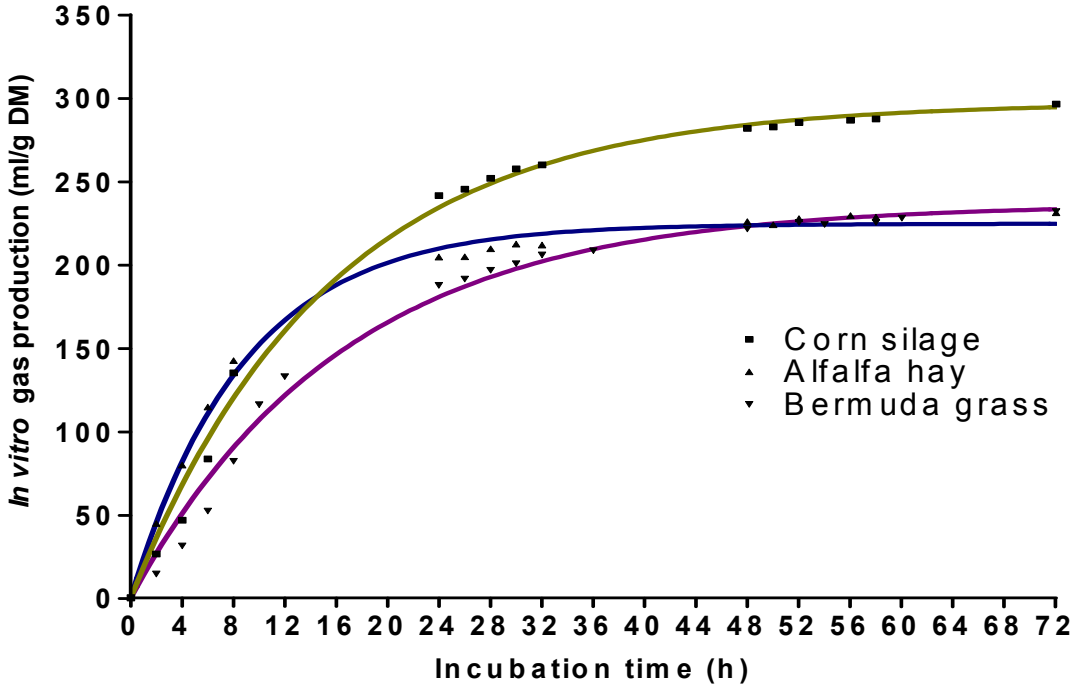

Figure 2. Theoretical gas accumulation from carbohydrate fermentation.