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Estimation of in Vivo Digestibility of Selected Albanian Feedstuffs by Hohenheimer Gas-Test and Cellulase Digestibility Method

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Abstract
Hohenheimer gas test digestibility (GAS) and Cellulase dry matter or organic matter digestibility (CDMD or COMD) of 53 Albanian feedstuff have been evaluated for their ability to estimate *in vivo* organic matter digestibility (OMD). The method developed by **Menke et al. (1979)** was used for gas production determination, while cellulase digestibility was investigated using the method of **DeBoever et al. (1986)**. The effects of GAS and CDMD/COMD on OMD were assessed using a backward multiple linear regression **(Sokal and Rohlf, 1995)**. A simple linear regression of OMD by the GAS, CDMD and COMD gave a regression equation of OMD = 30.240 + 0.738 GAS (R = 0.608, R^2 = 0.370), OMD = 25.868 + 0.565 CDMD (R = 0.628, R^2 = 0.394) and OMD = 25.529 + 0.602 COMD (R = 0.677, R^2 = 0.459) respectively. The Multiple linear regression of the *in vitro* variables (GAS, CDMD and COMD) gave a final model which used only COMD as a predictor. Digestibility value of the cellulase technique is nearer to the real value (OMD) than the value measured by the gas technique. The gas technique gave a significantly different value among replications especially for the grains, silage and straws groups, while the cellulase technique did not. The cellulase method is better than the gas technique in simplicity of the procedure, adjustment of the value to the real value (OMD) and in reproducibility.

Key words: Digestibility, gas-test, cellulase-technique, regression.

Introduction
For ration formulation purposes, beside nutrient requirement for animals, we also have to know the nutrient of feedstuffs. Although chemical analyses like the proximate and van Soest procedures have already been used widely, there are limitations of these methods to describe the feedstuff utilization by animals.

Determination of digestibility of dry matter *in vivo* gives a correction factor in terms of using feedstuffs in formulation but its determination consumes lots of money, time and labor. Furthermore, because of increasing public concerns elicited by the animal rights activists, the use of invasive surgical procedures for nutritional research becomes more difficult to justify.

Alternative procedures that are simple, reliable and inexpensive for predicting nutrient digestion of dietary feed ingredients in the rumen and small intestines are needed.

Various *in vitro* methods including the gas production and enzymatic methods have been used to predict digestion of feed ingredients. However, some results still contradict with the cellulase technique being reported as better than the gas production procedure.

This study was done with the general objective of establishing a feed digestibility and energy content database of selected Albanian feedstuffs. Opportunity was taken to appraise the precision of the gas test versus the cellulase method in estimating digestibility.

Materials and Methods

Fifty three samples of Albanian feedstuffs of known *in vivo* organic matter digestibility (OMD) were grouped as by-products (n = 6), fresh forages (n = 23), grains (n = 3), hays (n = 12), silage (n = 4) and straws (n = 5). The gas production (GAS), cellulase dry matter digestibility (CDMD) and cellulase organic matter digestibility (COMD) were determined to estimate the *in vivo* organic matter digestibility (OMD). Comparison of digestibility values of gas test and cellulase technique to OMD value was also performed. Reproducibility of the methods was assessed to determine the precision of the methods.

Gas production was analyzed according to the method of **Menke et al. (1979)**. Cellulase dry matter and organic matter digestibility were investigated using the same method as used by **DeBoever et al. (1986)**.

Results

Comparison between in vitro and in vivo values: The mean values of GAS, CDMD and COMD as compared to OMD are shown in Table 1. The differences obtained between values of cellulase (CDMD and COMD) and *in vivo* techniques are not significant for fresh forage, silage and grains groups. Digestibility values of the by-products group achieved by cellulase technique (CDMD) were 13.5 units higher than *in vivo* values, while for straws group, the values were lower by 20.2 units. The same pattern was also observed when COMD was used.

Table 1. Differences of means of digestibility as measured by *in vivo* and *in vitro* techniques.

Groups	n	OMD, %	CDMD, %		COMD, %		GAS (ml/200 mg)	
		(1)	(2)	(2 - 1)	(3)	(3 - 1)	(4)	(4 - 1)
By products	6	57.4	71.0	13.5	68.6	11.2	45.5	-12.0
Fresh forage	21	65.9	66.5	0.7	63.2	-2.7	41.6	-24.2
Grains	3	87.8	89.7	1.9	88.7	0.9	66.5	-21.3
Hays	11	53.8	61.9	8.1	57.3	3.5	44.3	-9.5
Silages	4	53.3	53.0	-0.3	53.7	0.4	38.9	-14.4
Straw	5	49.4	29.2	-20.2	27.0	-22.4	26.2	-23.2

The values obtained by the gas technique were lower than by the *in vivo* analysis. For fresh forages, grains and straws groups, the values differed greatly compared to the by-products, hays and silage groups.

Effect of in vitro digestibility on OMD: The Cellulase (CDMD and COMD) and the gas (GAS) techniques gave a significant correlation to OMD. The cellulase technique estimated OMD more accurately compared to gas technique. The coefficients indicated a positive relationship between (GAS, CDMD and COMD) and *in vivo* digestibility. The simple and partial linear regression of *in vitro* variables to estimate OMD is explained by the regression equations in Tables 2 and 3.

Table 2. Simple linear regression coefficient for predicting OMD by *in vitro* parameters

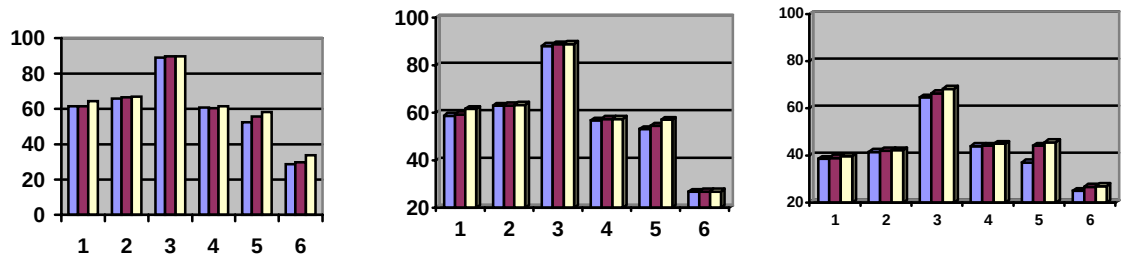
Model parameters	Coefficient	Probability	R	R^2
1. Constant (b_0)	25.529	0.000	0.677	0.459
COMD (b_1)	0.602	0.000		
2. Constant (b_0)	25.868	0.001	0.628	0.394
CDMD (b_1)	0.565	0.000		
3. Constant (b_0)	30.240	0.000	0.608	0.370
GAS (b_1)	0.738	0.000		

Table 3. Partial regression coefficients for predicting OMD using *in vitro* parameter (backward regression procedure)

Model parameters	Coefficient	Probability	R	R^2
1. Constant (b_0)	22.300	0.003	0.703	0.494
CDMD (b_1)	-0.224	0.667		
COMD (b_2)	0.564	0.326		
GAS (b_3)	0.408	0.174		
2. Constant (b_0)	23.023	0.002	0.701	0.491
COMD (b_1)	0.336	0.111		
GAS (b_2)	0.432	0.140		
3. Constant (b_0)	25.529	0.000	0.677	0.459
COMD(b_1)	0.602	0.000		

The multiple correlation coefficient of all *in vitro* variables was better than that of simple correlation. Combination of COMD, CDMD and GAS explained 49.4% of OMD variance compared to 45.9% by the COMD alone. However, the test of significance of the partial regression coefficient gave results which are not significant for the first and second model. Only the third model which used COMD alone is significant.

Reproducibility of in vitro method: Replication was embraced to study the reproducibility of the methods. Comparison of the *in vitro* methods in terms of their replication ability is shown in Figure 1. Analyses of variance among replications shows that both CDMD and COMD are not significantly different between replications. The gas technique, however, gave a big variation between replications for the grains, silage and straws groups.



CDMD COMD GAS

1 = By products, 2 = fresh forages, 3 = grains, 4 = hays, 5 = silage, 6 = straws

Figure 1. Reproducibility of the *in vitro* methods

Discussion

Comparison between in vitro and in vivo values: The cellulase technique gave a value near to the *in vivo* value when it was applied to the fresh forages, silage and grains groups. Application of the method on the by-products group gave an overestimation of digestibility (13.5 units) and on straws group underestimation (20.2 units). This underestimated value of straw was also reported by **Jones and Hayward (1973)**.

Application of gas technique for all groups of feedstuffs, gave a lower value compared to *in vivo* digestibility. The lower value (± 10 units) was found when the gas techniques were applied for the hays, silage and by-products groups. While other groups are lower by > 20 units.

These observations are explained by **Groot et al. (1997)** who state that the fermentation of organic matter of cell content is not linearly related to gas production kinetics. For the cell wall the kinetic of decline of degradable organic matter and fermentable organic matter were the same. The cell wall rich feed might have the value of gas nearer to the *in vivo* digestibility.

Effect of in vitro digestibility on OMD: In simple linear regression, the COMD and CDMD have a bigger correlation coefficient to OMD than GAS. Cellulase method compared to gas technique is superior in explaining OMD variance due to the fact that cellulase technique imitate *in vivo* digestibility more completely (fermentative and enzymatic steps) than gas technique (only fermentative step). A similar result was also found by **DeBoever et al. (1986)**.

The use of all *in vitro* variables in multiple linear regression (backward procedure) gave a final result which used only COMD variable in the model, other variables are held constant. The combination of *in vitro* variables (COMD, CDMD and GAS) gave the multiple

correlation coefficient up to 0.703. It means that about 49.4% of OMD variance is explained by this model. However, the significance test of partial regression coefficient gave a high value of probability for CDMD and GAS. Deleting of CDMD (which have the highest of probability value) from the model did not give a better significance value of others variables. After deleting the GAS variable, the rest of COMD variable gave the best model for OMD prediction. This observations is understandable, because COMD variables have the highest correlation coefficient to OMD than CDMD and GAS. The COMD explained the amount of organic matter digested *in vitro*, while OMD explained the amount of organic matter digested *in vivo*. The superiority of the cellulase over the gas technique was also reported by **Mannerkorpi (1992)**; **DeBoever et al. (1996a)**.

However, **Jones and Hayward (1973)**; **McQueen and van Soest (1974)**; **DeBoever et al. (1986)**; **Kuhla and Schmidt (1996)** found that the *in vitro* methods based on rumen liquor gave a better correlation than the enzymatic technique.

Reproducibility of in vitro method: The cellulase technique showed to be more reproducible than the gas technique. Similar results have been reported by **McQueen and van Soest (1974)**; **Kellner and Kirchgessner (1977)**; **DeBoever et al. (1996b)**. Evaluation of the cellulase digestion over several months showed to be reproducible both between different analytical batches and different operators. Analyses of replicated samples in three different batches and on different days using different reagents, gave a coefficient of variation of only 0.67%.

The gas technique has a big variation between replications, especially for the grains, silage and straws. The variation of gas production is due to the fluctuation of rumen microbes. The same results have been reported by **Wainmann et al. (1981)** and **van Der Meer (1983)**.

Conclusion: The cellulase technique gave a value near to the *in vivo* value when it is applied to the fresh forages, silage and grains groups. Application of the method on by products group gave an overestimation of digestibility (13.5 units) and on straws group, an underestimation (20.2 units). The gas technique gave lower values (± 10 units) for the hays, silage and by-products groups. For other groups, it gave more than 20 units lower than OMD value.

In vitro digestibility analysis showed that all parameters (COMD, CDMD or GAS) can be used in estimating OMD by simple linear regression. However, COMD has the highest correlation to OMD. Multiple linear regression of the *in vitro* variables gave a final model which used only COMD as a predictor.

The gas technique gave a significantly different value between replication especially for the grains, silage and straws groups, while the cellulase technique did not.

The cellulase method is better than the gas technique in simplicity of the procedure, adjustment of the value to the real value (OMD) and in reproducibility.

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