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Some methodological aspects for predicting whole plant maize digestibility from the "gas-test" technique

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SUMMARY - A good hay-inoculum appears as appropriate as, more practical and giving more reputable results than, a hay + concentrate or a maize silage-inoculum, in predicting the apparent digestibility of whole plant maize from the *in vitro* gas-test technique. The curve adjustment with 6 fermentation times (omitting the 96 h recording time) is as good as that with 7 fermentation times. The gas production after 24 h incubation is a better predictor than a+b of the mathematical adjustment. Less accurate than cellulase, it almost comes up with it when taken in combination with CP content. However, both the gas-test and the cellulase techniques are less efficient than the NIRS technique in predicting maize digestibility.

Key words: Digestibility, whole plant, maize, gas-test technique, *in vitro*.

RESUME - *"Quelques aspects méthodologiques pour la prédiction de la digestibilité du maïs plante entière à partir de la technique du "gaz-test". Un bon foin-inoculum semble aussi approprié et plus pratique, apportant des résultats plus répétables, qu'un concentré + foin inoculum ou un ensilage de maïs inoculum, pour la prédiction de la digestibilité apparente du maïs plante entière à partir de la technique du gaz-test* in vitro. *L'ajustement de la courbe avec 6 temps de fermentation (en omettant l'enregistrement de la 96ème heure) est aussi bon qu'avec 7 temps de fermentation. La production de gaz après 24 h d'incubation est un meilleur prédicteur qu'un ajustement mathématique de a+b. Moins précis que la cellulase, il est presque de même niveau lorsqu'il est étudié en combinaison avec la teneur en protéine brute. Cependant, aussi bien le gaz-test que la technique de la cellulase sont moins efficaces que la technique NIRS pour la prédiction de la digestibilité du maïs.*

Mots-clés : *Digestibilité, plante entière, maïs, technique du gaz-test,* in vitro.

Introduction

The "gas-test" technique is becoming popular for predicting the feeding value of forages. The present paper describes some methodological results of the work recently initiated in our Laboratory around the gas-test technique as a tool for predicting digestibility and intake of the main categories of forages, e.g., forage plants, whole plant maize and poor quality roughages, i.e., cereal straws and stovers.

Chapoutot *et al.* (1996) confirmed, on maize silages, the good correlations observed by Khazaal *et al.* (1993) on grasses and legumes hays between gas-test and *in situ* measurements. However Khazaal *et al.* (1995) observed relationships between *in vivo* data and prediction measurements less good with the gas-test technique than with the *in situ* technique.

As no studies considered yet the efficiency of the gas-test in predicting *in vivo* data in whole plant maize, and since we accumulated quite a large range of both *in vivo* and laboratory prediction measurements on this type of forage, we put emphasis in this work in answering the following methodological questions:

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(i) Is the nature of the rumen liquor (cellulolytic type *vs* amylolytic type) important for the prediction of maize digestibility?

(ii) In the case of a rapidly fermentable substrate like maize, is a late fermentation time (96 h) necessary for the mathematical curve adjustment of gas production, and are measurements of gas produced at a given time (without adjustment) as efficient predictors as curve adjustment?

We also took advantage of this opportunity to start comparing the "gas-test" with other available predictors of *in vivo* digestibility.

Materials and methods

Maize samples

19 out of the 234 samples of whole plant maize (the *in vivo* digestibility of which were measured with sheep) were chosen so that the range of variation of their organic matter digestibility (OMD) and of their crude protein content (CP) was large enough. Extreme values were respectively 67.1 to 78.9 p.100 for OMD and 53 to 94 g/kg DM for CP.

Crude fibre (CF), acid detergent fibre (ADF) van Soest and Wine (1967), starch (AMID), *in vitro* digestibility (IVD) Tilley and Terry (1963), cellulase digestibility (DCs) Aufrère (1983) and the spectrum in reflectance in the near infra-red spectrum (NIRS) had been determined at the Laboratory.

Diet of fistulates

The rumen liquors were obtained from two rumen-fistulated sheep and cows fed the following 150 g CP/kg DM diets:

1- sheep/hay:	1 kg/day of a cocksfoot regrowth hay.
2- sheep/hay/concentrate:	1 kg/day of 70% hay/30% concentrate (maize/soybean oil cake).
3- cow/maize silage:	6 kg (DM)/day of a maize silage supplemented with the adequate amount of urea to reach 150 g CP/DM.

The diets were fractionated into two equal meals per day (at 8 h for diets 1, 9 h for diets 2 and 3 and at 16 h for all diets). Rumen liquors were sampled 1 h after the morning meal for diet 1 and just before the morning meals for diets 2 and 3.

Gas-test technique

The technique used is that of Menke and Steingass (1988). It consists of 3 incubation runs per diet of 100 ml calibrated glass syringes containing each 30 ml of an inoculum (rumen liquor: buffer = 1:2) and 200 mg of duplicated maize samples or triplicate standard hay (that fed to the sheep) and no samples for the triplicate blanks. Volume (ml) of gas produced were measured at 3, 6, 12, 24, 48, 72 and 96 hours fermentation. pH, volatile fatty acids (VFA) and NH_3 were assayed in the 96 h residues in order to check the conditions of fermentation inside syringes. Data for gas production (ml) were fitted to the exponential equation

$$y = a + b\,(1 - e^{-ct})$$

of McDonald (1981), adapted, for "gas-test" and for the Microsoft Excel 5 software, by Anglaret (unpublished), on 7 and 6 (omitting the 96 h measurement) gas production records. y represents gas production at time t, (a+b) the potential gas production and c the rate of gas production.

Results and discussion

pH, NH_3 and VFA measurements (not provided in this paper) revealed satisfactory fermentation conditions in syringes with all inoculums.

As shown in Table 1, OMD prediction appreciated through the residual standard deviation of regression (syx) is only slightly more precise with "amylolytic" inoculums (syx=2.51 with maize silage) than with "cellulolytic" inoculum" (syx=2.63 with hay). Adding concentrate to the hay does not improve the prediction accuracy. The low loss in precision is largely compensated for the better repeatability of gas production data observed for maize and standard samples with the hay inoculum as compared with the hay/concentrate and, more over, the maize silage ones (Table 2, where the standard error -se- of (a+b) for maize samples is respectively 1.49, 1.64 and 3.48 ml with the hay, hay/concentrate and maize silage inoculums). This lower repeatability with inoculums coming from starch-included diets would deserve more investigation but likely advocates for the simpler diet, i.e.,.hay alone.

The prediction accuracy with 6 measurements is not different than with 7 measurements (Table 1). This is consistent with the fact that maize is a rapidly fermenting substrate and that, therefore, an extra 24 h incubation time from 72 to 96 h does not enables further fermentation which is almost completed at 72 and even at 48 h. It is then advisable stopping the kinetics at 72 h (even earlier as did Chapoutot *et al.,* 1996) in order to save time (and labour); nevertheless it would be interesting to check whether an extra recording time (and point for the curve adjustment) at the beginning of the fermentation process (e.g., 9 h) would improve the mathematical model.

The quantity of gas produced after 24 h is the best prediction parameter of those using the gas produced at a given time (Table 1). It is even better, whatever inoculum, than (a+b) 6 or 7 measurements (syx=2.51 and 2.64 and 2.63, respectively in the case of hay inoculum). This observation is important and would deserve more attention since, as far as maize substrates are concerned, it would imply a considerable reduction of the work load.

Table 1. Prediction accuracy of *in vivo* apparent organic matter digestibility (%) of 19 whole plant maizes by sheep from characteristics (a+b) of cumulative *in vitro* gas production calculated either with 7 or with 6 (omitting the 96 h measurement) fermentation times or from the gas produced (ml/200 mg DM) at 12, 24, 48 or 72 hours of fermentation, according to the diet of fistulated animals

Predicting criteria	Fistulated diet					
	Sheep/hay		Sheep/hay/concentrate		Cow/maize silage	
	r	syx	r	syx	r	syx
Gas-test						
a+b (7 measurements)	0.545	2.63	0.528	2.66	0.596	2.52
a+b (6 measurements)	0.537	2.64	0.507	2.63	0.599	2.51
12 h	0.229	3.05				
24 h	0.598	2.51	0.587	2.54	0.650	2.38
48 h	0.579	2.55	0.569	2.57	0.603	2.50
72 h	0.551	2.62	0.540	2.64	0.598	2.51

Table 2. Variability, expressed as standard error (se), of *in vitro* gas production (ml/200 mg DM) observed on 3 incubation runs (blanks and standards triplicate and maize samples duplicated in each run) according to the diet of the fistulated animals

Diet	Sheep/hay		Sheep/hay+concentrate		Cow/maize silage	
Gas production considered	24 h	(a+b)	24 h	(a+b)	24 h	(a+b)
Blanks	1.65	1.39	2.40	3.19	1.50	1.80
Standards maize samples	1.19	1.33	1.21	2.61	1.57	3.02
Mean (n=19)	1.43	1.49	1.61	1.64	3.02	3.48

When restricting on the hay inoculum (Table 3), in view of the former reason, gas 24 h is only slightly less good (syx=2.51) than IVD (syx=2.40) but inferior to DCs (syx=1.96). The association of an extra predictor (either of cell content type like CP or starch, or of cell-wall type like CF or ADF) improves the prediction accuracy except with starch. Gas 24 h, CP (syx=2.04) and gas 24 h, CF (syx=2.04) then, almost come up with DCs, CP (syx=1.83) and are both superior to IVD, CP (syx=2.41). Why adding CP to IVD does not improve the prediction as compared with gas-test would deserve more explanation.

At last, NIRS might (but it is necessary to have at least 50 samples to be allowed to calculate a regression equation with NIRS) enable predicting the 24 h gas volume and it is likely the best method to predict maize digestibility (syx=1.02).

Table 3. Prediction accuracy of *in vivo* apparent organic matter digestibility (OMD) (%) of 19 whole plant maizes by sheep from *in vitro* gas production (ml/200 mg DM) at 24 hours of fermentation (with the hay rumen liquor), alone or associated with concentration (% DM) of crude protein (CP) or acid-detergent fibre (ADF) or crude fibre (CF) or starch (AMID), or from *in vitro* OMD (IVD) (Tilley and Terry, 1996) or cellulase digestibility (DCs) (Aufrère and Michalet-Doreau, 1983), alone or associated with CP, or from the Near Infra Red Spectrometry (NIRS).

Predicting criteria	r	syx
Gas 24 h	0.598	2.51
Gas 24 h, CP	0.775	2.04
Gas 24 h, ADF	0.769	2.06
Gas 24 h, CF	0.775	2.04
Gas 24 h, AMID	0.728	2.21
IVD	0.644	2.40
IVD, CP	0.666	2.41
CDs	0.781	1.96
CDs, CP	0.825	1.83
NIRS (stepwise)	0.880	1.02

Conclusions

The "gas-test" is an interesting but somehow sophisticated technique which deserves being perfectly mastered. More investigation on more samples is required regarding ways and means to reduce the work load (by reducing the number of measurement time and, even, taking only one of them) and to improve the accuracy of OMD prediction that, for the time being, remains lower than with NIRS.

Further work is also necessary, and being carried out, on the prediction of voluntary intake.

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