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A comparison of alternative sources of inocula in an in vitro digestibility technique

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Summary — In this paper we make the comparison between sources of inocula for an in vitro digestibility method based on that of Tilley and Terry (1963) as modified by Alexander and McGowan (1966). The best results for the prediction of the in vivo digestibility were obtained using the traditional method of rumen liquor from fistulated sheep. However, with the two alternative methods, one using rumen liquor from slaughtered cattle, and another, a sheep faeces suspension as inocula sources, we obtained results significantly correlated with in vivo digestibility at the 5% level, although with lower correlation coefficients. The method that uses rumen liquor from slaughtered cattle is, in our opinion, a valid alternative to the traditional method.

in vivo digestibility / in vitro digestibility / sources of inocula / alternative methods

Résumé — Comparaison de sources alternatives d'inoculum pour la mesure de la digestibilité in vitro. *Dans cet essai, nous avons comparé l'effet de trois sources d'inoculum pour la détermination de la digestibilité in vitro par la méthode de Tilley et Terry (1963) modifiée par Alexander et McGowan (1966). Les sources d'inoculum étaient : du jus de rumen de moutons fistulés (méthode traditionnelle), du jus de rumen de vaches abattues, prelevé à l'abattoir (méthode de Nikolic et al,1987) et des fèces de moutons (méthode proposée par El Shaer et al, 1987). Au total, 24 échantillons de graminées ont été employés : avoine, ray-grass d'Italie, ray-grass anglais et maïs, à trois stades de développement, en vert et après ensilage. Le coefficient de digestibilité in vivo des fourrages (tableau I) a été déterminé sur six moutons adultes, mâles, alimentés ad libitum pendant une période de 21 jours, 14 jours d'adaptation et 7 jours de mesure. Les meilleurs résultats pour la prévision de la digestibilité in vivo furent obtenus avec la méthode traditionnelle, avec du jus de rumen prélevé sur des moutons fistulés. Des liaisons significatives (niveau 5 %) avec la digestibilité in vivo ont aussi été obtenues avec les méthodes alternatives, mais les* R^2 *sont plus faibles (tableau III). La méthode qui utilise du jus de rumen de vaches abattues est cependant une bonne alternative.*

digestibilité in vivo / digestibilité in vitro / sources d'inoculum / méthodes alternatives

INTRODUCTION

Digestibility is the most important biological measure of a forage. It can be measured in vivo, but this method is laborious, consumes large amounts of forage and requires a relatively large number of animals. Therefore, several laboratory methods have been proposed for this estimation. The in vitro methods attempt to simulate the natural ruminant digestive processes. The method of Tilley and Terry (1963) modified by Alexander and McGowan (1966) can be considered the most usual, presenting however, one disadvantage, in that it requires fresh inocula from permanently fistulated animals. With the objective of avoiding this obstacle, several authors have proposed alternative methods. Until recently these methods were mainly chemical, physical and enzymatic, in which the cellulolytic function of the rumen liquor is substituted by an enzymatic preparation. More recently, however, other laboratory techniques have been proposed. A modified two-stage technique is based on the classic procedure (Tilley and Terry, 1963), but employing bovine rumen fluid from a slaughterhouse (Nikolić et al, 1987), and the method proposed by El Shaer et al (1987) based on the use of faecal microorganisms contained in a filtered suspension of sheep faeces. The microbial methods have clear advantages over the enzymatic techniques in the prediction of the in vivo digestibility (Omed et al, 1989).

Our objective is to determine if the alternative sources of inocula can substitute for the traditional source (rumen liquor from fistulated sheep) in the determination of in vitro digestibility by the method of Tilley and Terry (1963) modified by Alexander and McGowan (1966), and therefore go beyond the inconvenience of the method: the need for fistulated animals. The use of alternative sources of inocula make a significant contribution to the welfare of the laboratory animals, and simplify the method because it is much easier to obtain sheep faeces or rumen liquor from slaughtered cattle than rumen liquor from fistulated sheep.

MATERIALS AND METHODS

Forage samples of 24 graminaceae (oat, Italian ryegrass, perennial ryegrass and maize) in three stages of growth, fresh and ensiled, with a known chemical compositon and in vivo digestibility were employed. The forages were harvested with a precision-chop machine and chopping length was 15 mm. Approximately 1.5 tonnes of each fresh forage was frozen at –15 °C, and the other portion (1.5 tonnes) was ensiled. The silos were opened after 60 days and the silage was frozen at –15 °C.

Six adult Romney-Marsh male sheep per treatment, with body weights of approximately 15 $kg^{0.75}$, were used for the in vivo digestibility determinations. The digestibility trials lasted 21 day: a 14-day adaptation period and a 7-day collection period. The animals were fed ad libitum twice daily, at 9 00 and 17 00 hours. The quantities of feed offered were the previous days consumption amounts plus 10%. The ranges of in vivo dry matter digestibilities are shown in table I.

For the laboratory method, forage samples were dried at 65 °C for 48 hours and milled through a screen of 1.0 mm.

The reference method used was that proposed by Tilley and Terry (1963) and modified by Alexander and McGowan (1966): 0.5 g of each sample was weighed in triplicate and incubated for 48 hours with a mixture of 40 ml of McDougall (1948) buffer solution and 10 ml of inocula saturated with CO_2. At the end of the first fermentation stage, microbial activity was stopped with 2.2N HCl until a pH of 1.2 was achieved. This was followed by an incubation period of 48 hours with 50 ml of a solution of acid pepsin. The residue obtained after filtration in a G_2 crucible was dried at 105 °C and then ashed at 550 °C.

This method was employed with rumen liquor obtained from three rumen fistulated adult Romney-Marsh sheep that were fed ad libitum a standard diet of mid-quality hay and 200 g of compound feed per day (table II). We also used the inocula source reported by Nikolić et al (1987); rumen contents were removed from six healthy cattle immediately after the slaughter, and stored in thermolagged containers. After straining

Table I. Forages used and the range of their in vivo dry matter digestibilities.

Forages	*No of samples*	*Range of in vivo digestibility (%)*
Oat	6	51.4–66.5
Italian ryegrass	6	56.4–71.2
Perennial ryegrass	6	49.3–74.2
Maize	6	55.5–63.8

Table II. Mean chemical composition of the feeds given to the sheep.

Feed	*DM (%)*	*100 g DM*					
		CP	*EE*	*Ash*	*CF*	*Ca*	*P*
Hay	89.0	17.0	1.3	11.6	26.0	1.7	0.3
Compound feed	89.0	18.9	3.0	12.9	19.3	1.1	0.4

DM: dry matter; CP: crude protein; EE: ether extract; CF: crude fibre.

through four layers of gauze, the rumen liquor was mixed with McDougall's buffer solution, in porportion 4:1, and saturated with CO_2. Since previous dietary history was unknown, the use of a large number of 'donor animals' reduced the variability of the inocula microbial activity.

The other method used was that proposed by El Shaer et al (1987): the source of inocula is not rumen liquor but sheep faeces. A 50 g sample of wet sheep faeces were collected within 1 hour of voiding from three sheep that were maintained on a diet of average quality hay and 200 g of compound feed (table II). The faeces were macerated and mixed with 50 ml of McDougall's buffer solution, which had previously been saturated with CO_2. The mixture was filtered and then made up to 300 ml with buffer solution, and the pH was adjusted to 6.8.

A simple linear regression and paired *t*-tests were used to compare the relationship between in vitro and in vivo digestibilities. The data were subjected to analysis of variance (Anova) based on the results of the dry matter digestibility. Comparisons were made for the three stages of growth, and two forms of conservation and were tested for the interaction between species and growth, species and conservation, and growth and conservation. The multiple comparison of the means were made using the *t*-test (Steel and Torrie, 1980).

RESULTS

The equations of regression between the in vitro digestibility of dry matter and the in vivo determinations for all forages are presented in table III and for fresh and ensiled forages separately in table IV.

The relationships between in vitro digestibility determined by the method with sheep rumen liquor, with slaughtered cattle rumen liquor and with sheep faeces, and the in vivo dry matter digestibilities, respectively, are shown in figures 1, 2 and 3.

The variance analysis of the results of the dry matter digestibility obtained by in

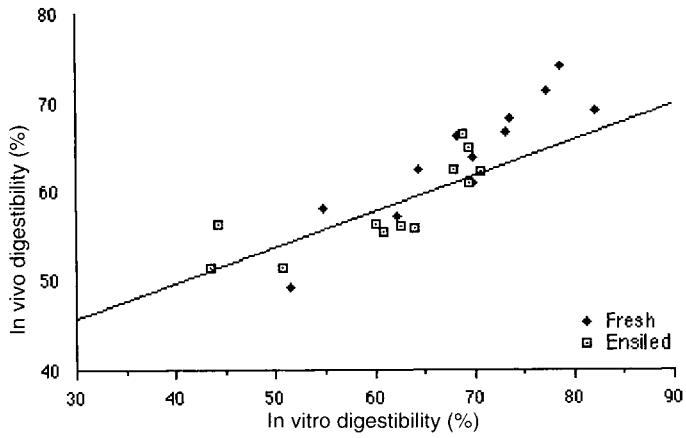

Fig 1. Relationship between the dry matter digestibility estimated in vitro with sheep rumen liquor and determined in vivo.

Table III. Relationship between the dry matter digestibility estimated in vitro and determined in vivo.

Method	*Constant*	*Variable*	n	R^2	*RSD*
Sheep rumen liquor	25.48	0.55	24	0.76*	3.35
Cattle rumen liquor	42.01	0.35	24	0.42*	5.14
Sheep faeces	35.38	0.33	24	0.33*	5.52

n: number of samples; R^2: coefficient of determination; RSD: residual standard deviation. * ≤ 0.05.

Table IV. Relationship between the dry matter digestibility estimated in vitro and determined in vivo for the two groups of forages.

Method	*Forage*	*Constant*	*Variable*	n	R^2	*RSD*
Sheep rumen liquor	Fresh	17.03	0.68	12	0.85*	2.83
	Ensiled	33.78	0.40	12	0.62*	3.19
Cattle rumen liquor	Fresh	34.80	0.49	12	0.63*	4.39
	Ensiled	54.29	0.08	12	0.02	5.15
Sheep faeces	Fresh	37.11	0.35	12	0.33*	5.89
	Ensiled	33.88	0.31	12	0.43*	3.94

n: number of samples; R^2: coefficient of determination; RSD: residual standard deviation. * ≤ 0.05.

vivo and in vitro methods for the three stages of growth and two forms of conservation are presented in table V.

DISCUSSION

Based on the coefficients of determination (R^2), we can assume that satisfactory estimates of the in vivo digestibility values were obtained by all three methods. Judging by the residual standard deviation, the traditional method gave the most precise estimate. We verified that the alternative methods to the Tilley and Terry (1963), modified by Alexander and McGowan (1966), with rumen liquor from fistulated sheep gave results significantly correlated ($P = 0.05$) with in vivo digestibility. The method that used rumen liquor from slaughtered cattle was highly correlated with the in vivo organic matter digestibility (r value = 0.969) and corroborated the findings of El Shaer et al (1987), who observed an r value of 0.98 for the correlation between the results of the in vivo dry matter digestibility and the results of the sheep faeces method.

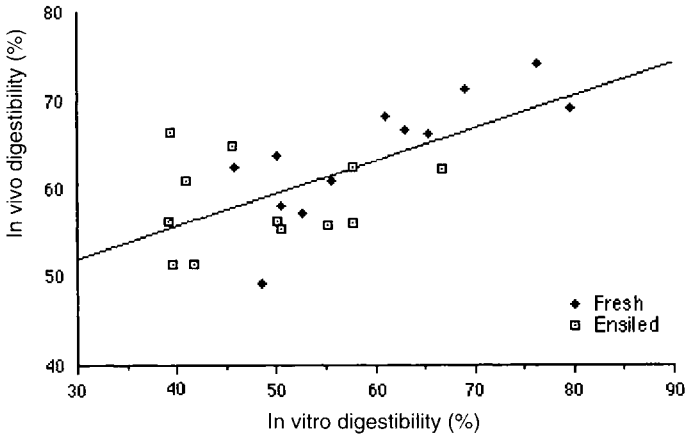

Fig 2. Relationship between the dry matter digestibility estimated in vitro with slaughtered cattle rumen liquor and determined in vivo.

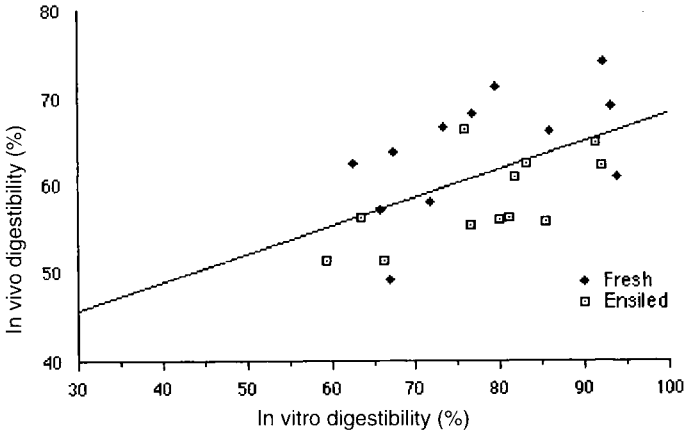

Fig 3. Relationship between the dry matter digestibility estimated in vitro with sheep faeces and determined in vivo.

Table V. Variance analysis of the results of the dry matter digestibility obtained by in vivo and in vitro methods for the three stages of growth and two forms of conservation.

	n	*In vivo* (%)	n	*Sheep rumen liquor (%)*	*Cattle rumen liquor (%)*	*Sheep faeces (%)*
Species						
Oat	36	60.16[a]	18	62.22[a]	53.33[a]	76.46[a]
Italian ryegrass	36	64.04[b]	18	65.99[a]	51.16[a]	78.39[a]
Perennial ryegrass	36	61.42[ab]	18	61.46[b]	61.46[b]	81.16[a]
Maize	36	59.09a	18	64.91[ab]	51.21[b]	74.87[a]
Growth						
Young	48	64.05[b]	24	71.12[c]	59.83[b]	81.29[b]
Normal	48	64.05[b]	24	68.72[b]	48.75[b]	81.37[b]
Later	48	55.42[a]	24	55.05[a]	47.27[a]	70.50[a]
Conservation						
Fresh	72	63.91[b]	36	68.93[b]	59.83[b]	77.45[a]
Ensiled	72	58.45[b]	36	60.99[a]	49.75[a]	77.99[a]
SD		5.23		2.14	3.44	10.01
S Species (Sp)		*		*	*	NS
Growth (Grow)		*		*	*	*
Conservation (Con)		*		*	*	NS
Sp x Grow		*		*	*	*
Sp x Con		NS		*	*	NS
Grow x Con		*		NS	*	NS

n: number of observations; SD: standard deviation; S: level of significance ≤ 0.05; NS: not significant. [abc] Where similar letters on the same column are indicated, there are no significant differences between means.

The method that used the rumen liquor from slaughtered cattle gave an R^2 lower than that of the traditional Tilley and Terry (1963), modified by Alexander and McGowan (1966), in the prediction of the in vivo digestibility (table V). The greatest advantage of the method using rumen liquor from slaughtered animals was the avoidance of fistulated animals as the inocula source and the ease and low cost of the procedure. The results of the method that used the faeces suspension as the source of inocula had the lower relationship with the in vivo digestibility and a higher standard deviation (table V). This technique is, in our opinion, not recommended for the prediction of in vivo digestibility for this reason and because the results are much higher than those determined by the in vivo method (table V). This occurs with the methods that used faeces microorganisms because of the difference between the rumen and the faecal microbial population and their ability to break down cell walls.

The regression equations between the in vitro digestibility of dry matter and the in vivo determinations show that the method that used the sheep rumen liquor obtained a higher R^2 with the fresh forages than with ensiled forages (table IV). The method that used the rumen liquor from slaugtered cattle presented a higher R^2 from the fresh for-

ages but did not give a significant correlation at the level 5% from the ensiled forages (fig 2). The method that used sheep faeces also gave results significantly correlated with in vivo digestibility from fresh and ensiled forages. The relationships obtained were generally better for fresh forage than for ensiled forage. Vanderhaege and Biston (1987) and Omed et al (1989) obtained the same relationship with a pepsin-cellulase method in a trial that compared faeces liquor-pepsin, rumen liquor pepsin and pepsin-cellulase, three techniques for the prediction of the dry matter digestibility. Barber et al (1984), using a rumen liquor-pepsin method, reported a higher R^2 with silages than with fresh forages; Aufrère (1982), with a pepsin-cellulase method, obtained r values of 0.94 and 0.96, respectively, for fresh grass and ensiled forages.

Our results should be confirmed with the repetition of the trial and the utilization of a blind test population of forages. Clary et al (1988) reported that when a multiple donor approach is combined with the use of standard reference forages, the most stable and reliable in vitro digestion values should be obtained. The use of control samples in each series is thus necessary to permit comparison between laboratory results (Aufrère and Michalet-Doreau, 1988).

CONCLUSION

The two-stage procedure for the determination of the in vitro digestibility described by Tilley and Terry (1963), and modified by Alexander and McGowan (1966), with rumen liquor from fistulated sheep as inocula gave the best results for the prediction of the in vivo digestibility. However, we also had good results with the same method when the source of inocula was rumen liquor from slaughtered cattle. The method that used faeces suspension as inocula had the lowest correlation with the in vivo digestibility and a higher standard deviation.

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