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Prediction of forage digestibility from some laboratory procedures[1]

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Summary

A description is given of the accuracy with which digestibility is predicted from some laboratory procedures of van Soest, using 106 Dutch forages.
The in vitro procedure gave fairly accurate results, whereas the results obtained with the 'summative equation' (a chemical procedure) were less satisfactory. Tracing the causes of the latter showed that the relationship between the digestibility of the cell-wall constituents and the lignin content was rather bad, possibly due to the rather low lignin contents of the forages, enabling plant silica and soil contamination to become more important.
The percentage of apparently digested cellular contents was closely related to the percentage cellular contents, but the lines for the Wageningen and United States samples deviated from those on the Hoorn samples and it is suggested that this might be caused by the different physiology of the microflora in the rumen.

Introduction

Since its development about 100 years ago, the Weende system of feed analysis has been a widely accepted procedure for measuring chemical composition and predicting digestibility of forages. The crude-fibre content is generally used as a reference in this. The Weende procedure has been very persistent despite the faulty results which were frequently obtained. This inaccurate calculation of digestibility from the crude-fibre content has been a large handicap for the first author in his research into the influence of environmental conditions on forage quality (Deinum, 1966).
In recent times, however, some procedures have been developed which enable more accurate prediction of forage digestibility, and in many countries and institutes the Weende system has already been replaced by these new techniques. Tilley and Terry (1963) and van Soest et al. (1965, 1966) have been especially successful in developing

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better techniques. In this paper the results of the van Soest procedures with a group of Dutch forages will be described.

The procedures of van Soest are based on the new information which detailed research disclosed about the digestion processes in the rumen and intestines of ruminants. The ingested forage is attacked by bacteria and protozoa in the rumen so fiercely that only a part of the cell walls remains undigested, leaving no possibilities for further digestion in the following part of the digestive tract. During this rumen digestion the bacteria build up their own bodies under excretion of fermentation end products like acetic, propionic and butyric acid. These bacteria and protozoa are attacked in the intestines, but the rather weak animal enzymes are only able to dissolve the inner parts, leaving the bacteria cell walls undigested. Thus the faeces consist of the undigested parts of the plant cell walls, the bacteria cell walls and endogenous substances excreted into the intestines.

The true digestibility is called that part of the food which is actually digested; the apparent digestibility is the weight loss between mouth and rectum, which equals the true digestibility minus the bacteria cell walls and endogenous excretion.

This paper will describe how accurately forage digestibility is predicted with the procedures based on this physiological background. Two procedures have been developed, a chemical method and a biological in vitro method. The chemical analysis may explain digestibility if the right components are analysed whereas it may be expected that a correct in vitro measurement will be better if there are any chemical factors left unanalysed.

Experimental

Description of the procedures

The chemical approach. The first important treatment to be made is the separation of the forage into cellular contents (% CC) and cell-wall constituents (% CWC). The cellular contents consist of protein, starch, sugars, minerals, fats, organic acids and some substances of minor importance. The cell-wall constituents are cellulose, hemicellulose, lignin, cutin, soil contamination and sometimes silica metabolized by the plant. The cellular contents are almost completely digestible (D_{CC} is about 98%), whereas the digestibility of the cell-wall constituents (D_{CWC}) is mainly determined by its lignin content (L × 100/CWC). However, the correlation between D_{CWC} and lignin is usually somewhat better when the lignin content of acid-detergent fibre (L × 100/ADF) is used, in which ADF is the sum of cellulose + lignin + plant silica + soil contamination. In a large group of samples from different origin van Soest and Wine (1968) found $D_{CWC} = 181 - 96.6 \log (L \times 100/ADF)$. The true digestibility of a sample is then the sum of the digestible cellular contents and the digestible cell-wall constituents:

$D_{true} = 0.98\ CC + CWC\ [1.81 - 0.966 \log (L \times 100/ADF)]$

This formula is called the 'summative equation'. ADF, CWC and L are determined according to van Soest (1963) and van Soest and Wine (1967, 1968) respectively.

The biological approach. The digestibility (D_{vitro}) can be determined as well by treating the forage sample with rumen fluid, after which the remainder of the plant cell-

wall constituents is determined (van Soest et al., 1966). This procedure will be referred to as the in vitro method.
In both cases the true digestibility is calculated. After subtraction of the bacterial cell wall plus endogenous excretion (further called bacterial excretion) the apparent digestibility is found. The latter calculation can be done if faeces samples are available for the determination of the bacterial excretion (= 100 — % CWC). In only a few cases were these samples present; therefore, the calculated true digestibility had to be correlated directly with the apparent digestibility in vivo (D_{vivo}) in dry matter. All the analyses were done by the first author in the laboratory of the Animal Husbandry Research Division, A.R.S., Beltsville, Md, U.S.A.

Description of the samples
The techniques mentioned above were able to be tested on 136 samples of known digestibility in vivo. Of these 124 were generously provided by the Institute for Livestock Feeding and Animal Research, Hoorn, the Netherlands, and 12 by the Department of Animal Physiology, Agricultural University, Wageningen, the Netherlands (the faeces samples of these 12 grasses were available as well). 106 samples will be described here. These were subdivided into four groups, as mentioned in Table 1.
The remaining 30 samples were pellets, meals and samples from non-grass or legume forages. The pellets and meals were omitted because of heat damage that occurred during drying, pelleting and grinding, while the sixth group was discarded because of severely deviating material in many samples.

Table 1 Brief description of the samples used

	Digestibility in vivo			
	number	*average*	*lowest*	*highest*
Hay	56	63.8	53.3	73.7
Grass	24	72.8	59.6	84.6
Grass silage	11	66.5	59.9	72.8
Legumes	9	58.9	53.6	68.2

Results

The in vitro procedure
The group of hays, grasses and grass silages will be considered as one group to which the legumes will be added. Using regression analysis the following results are found:
without legumes:

$$D_{vivo} = 1.28\ D_{vitro} - 44.8\ (n = 97;\ r^2 = 0.797;\ RSD = 2.77) \quad (1)$$

with legumes:

$$D_{vivo} = 1.07\ D_{vitro} - 25.9\ (n = 106;\ r^2 = 0.780;\ RSD = 2.99) \quad (2)$$

From these equations and from Fig. 1 it can be learned that the in vitro system gives a good correlation and a fairly low residual standard deviation (RSD). Similar

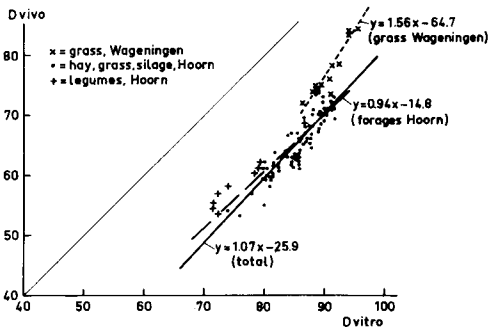

Fig. 1 Relationship between the apparent digestibility of dry matter in vivo (D_{vivo}) and the true digestibility in vitro (D_{vitro}) of 106 Dutch forages

residual standard deviations were found by van Soest et al. (1966) on New England samples, although the regression equation in their samples was $D_{vivo} = 0.96\ D_{vitro} - 10.4$. The average bacterial excretion ($= D_{vitro} - D_{vivo}$) calculated as percentages of intake was in these samples about 20 units, which is high compared to the 13 units found by van Soest et al. (1966). However, the latter value was found in the 12 grasses from Wageningen, and omitting this group from the Hoorn samples reduced the residual standard deviation of the equations 1 and 2 to about 2.0–2.2. These separate regression equations were:

forages Hoorn:

$$D_{vivo} = 0.94\ D_{vitro} - 14.8\ (n = 94;\ r^2 = 0.810;\ RSD = 2.18) \quad (3)$$

grass Wageningen:

$$D_{vivo} = 1.56\ D_{vitro} - 64.7\ (n = 12;\ r^2 = 0.860;\ RSD = 1.73) \quad (4)$$

This matter will be discussed later on.

The 'summative equation'

Using the 'summative equation' (S) the following results were obtained:

without legumes:

$$D_{vivo} = 0.78\ S - 0.1\ (n = 95;\ r^2 = 0.451;\ RSD = 4.24) \quad (5)$$

$$D_{vivo} = 0.87\ S - 1.19\ AD_{ash} - 2.9\ (n = 95;\ r^2 = 0.645;\ RSD = 3.43) \quad (6)$$

with legumes:

$$D_{vivo} = 0.76\ S - 1.8\ (n = 104;\ r^2 = 0.524;\ RSD = 4.12) \quad (7)$$

$$D_{vivo} = 0.86\ S - 1.11\ AD_{ash} - 2.2.\ (n = 104;\ r^2 = 0.676;\ RSD = 3.41) \quad (8)$$

These equations show that the 'summative equation' itself (equations 5 and 7) did not give very accurate results, not much better than the old crude-fibre method. However, many samples contained a high percentage of soil contamination and plant silica, which is not dissolved in the ADF determination. Therefore, including this acid-detergent-insoluble ash (AD_{ash}) in the regression analysis improved the system considerably (equations 6 and 8), although the residual standard deviation did not become as low as in the in vitro system. These results agree fairly well with the findings of van Soest and Jones (1967, 1968) on samples, from the midwestern areas of the United States, which usually contain plant silica. In their samples the residual standard deviation was 5.8 before and 3.6 after correction for the silica content.

Discussion

What may be the cause of these not yet ideal results of the 'summative equation'? It must be repeated that the chemical procedures will only give a good estimate if all the chemicals controlling digestibility are determined in the right way.
There are three possible sources of errors, and by combining the in vitro data and the chemical data it is possible to distinguish between them and to trace where the biggest errors are made. However, this requires closer examination of what is happening with the food during digestion. Firstly, there is the digestion of the cell-wall constituents, secondly, the digestion of the cellular contents, and thirdly, the bacterial excretion may cause errors.

The digestion of the cell-wall constituents
How accurately the digestibility of the cell-wall constituents can be estimated from the lignin content has to be investigated. This makes it necessary to predict D_{CWC} of our samples, which can be done in the following way.
In the in vitro procedure the quantity of the undigested plant cell-wall constituents is determined. If the percentage of CWC of the forage is known, the D_{CWC} in vitro can be calculated:

$$D_{CWC} = 100 \times \left(1 - \frac{\text{undigested CWC}}{\text{CWC in forage}}\right)$$

After this it is necessary to prove that this D_{CWC} in vitro equals D_{CWC} in vivo. For this purpose 31 forages of known faeces composition and D_{CWC} in vivo were available. The results are shown in Fig. 2, from which it is clear that D_{CWC} in vitro agreed very well with D_{CWC} in vivo. This good agreement showed furthermore that our digestion in vitro was as efficient as the rumen digestion in vivo.
Knowing this good agreement, there is justification for relating digestibility in vitro of the cell-wall constituents with lignin content and with other parts of the cell walls.

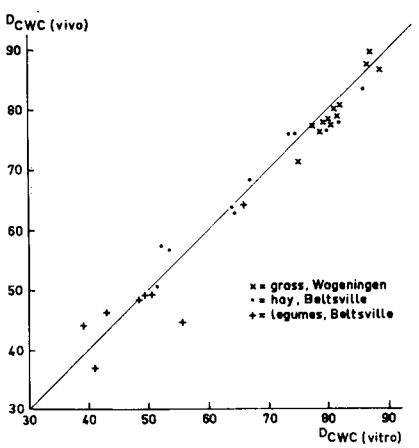

Fig. 2 Relationship between the digestibility in vivo and in vitro of the cell-wall constituents of 19 American and 12 Dutch forages

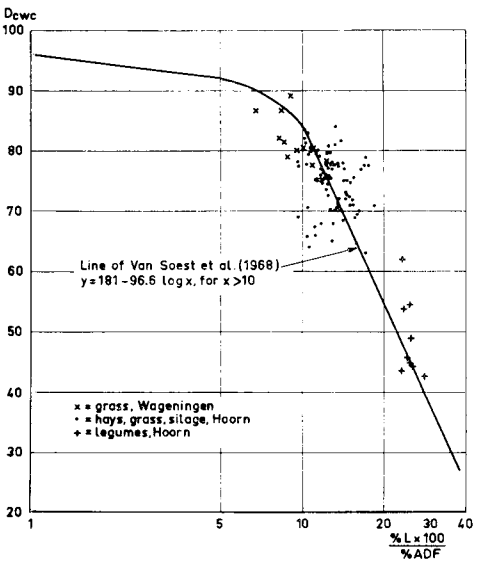

Fig. 3 Relationship between the in vitro digestibility of the cell-wall constituents (D_{CWC}) and the lignin content of acid-detergent fibre (L × 100/ADF) of 106 Dutch forages

The results are shown in Fig. 3, whereas the calculated equations are:
without legumes:

$$D_{CWC} = 109.3 - 30.9 \log (L \times 100/ADF) \quad (n = 97;\ r^2 = 0.226;\ RSD = 4.61) \quad (9)$$

$$D_{CWC} = 115.3 - 33.5 \log (L \times 100/ADF) - 0.49\ (AD_{ash} \times 100/CWC) \quad (n = 97;\ r^2 = 0.375;\ RSD = 4.17) \quad (10)$$

with legumes:

$$D_{CWC} = 143.2 - 62.7 \log (L \times 100/ADF) \quad (n = 106;\ r^2 = 0.611;\ RSD = 5.75) \quad (11)$$

$$D_{CWC} = 148.6 - 65.0 \log (L \times 100/ADF) - 0.43\ (AD_{ash} \times 100/CWC) \quad (n = 106;\ r^2 = 0.646;\ RSD = 5.49) \quad (12)$$

These results show a rather poor correlation in comparison with the results of van Soest and Wine (1968). Including AD_{ash} improves the system only slightly. However, the points are rather regularly scattered around van Soest's line. It is clear from these results that the estimate of cell-wall digestibility from the L/ADF value has been quite erratic, and this will explain in large part the unsatisfactory results of the summative equation.

It is certain that lignin is the predominant factor controlling digestibility and that the relationship between D_{CWC} and (L × 100/ADF) is very good in essence, as is shown by van Soest et al. (1965, 1968). However the average lignin content of these Dutch samples is much lower than that of the American samples, which makes it possible that other substances e.g. plant silica and cutin become more important than lignin in inhibiting digestion.

Acid-detergent-insoluble ash is considered here as an inert material and for the most part this is correct, for many samples contained fair amounts of undigestible soil contamination. However, van Soest and Jones (1967, 1968) have discovered that many grass species are able to metabolize SiO_2 from the soil, precipitating it into the cell

walls. There it inhibits digestion to the same extent as lignin does. Initially this silica was not considered important in our samples, but later on plant silica became visible in many samples after ashing. Afterwards, the presence of plant silica was not surprising for most of the Hoorn forages were collected from clay soils on which grasses metabolize silica ('t Hart, 1945). However, attempts to separate soil contamination and plant silica failed, because of the fact that during dissolving the opaline plant silica in NaOH a variable amount of the glass filter crucible was dissolved as well. Consequently only a suggestion can be made that a large part of the variation on the left-hand side of the line in Fig. 3 may be caused by plant silica.
It is also possible that the lignin procedure does not exactly measure the content of the true lignin which will inhibit digestion of the plant cell-wall constituents. So too high lignin contents are found on the right-hand side of the line. These deviations originate mainly from some very immature grass samples. This is a predominant forage in the Netherlands during the growing season, and some digestion of the lignin will occur. For example, digestibility of lignin in vivo varied from 8–47 % in the 12 grass samples from Wageningen. Some of the results from these samples are described by Deinum et al. (1968). Moreover it was found in these samples that the relationship between D_{CWC} and L/ADF was rather poor, while the relationship between D_{CWC} and undigested L/ADF of the forage was very sharp.
These results may suggest two things. Firstly, due to its variable digestibility, lignin contents may have led to the unsatisfactory results in estimating digestibility of the very immature grasses. Secondly, the presence of soil contamination and plant silica may have disturbed the system even more.

Apparent digestion of cellular contents
After this two other possibilities of errors in the summative equation remain, but they have to be treated together because of the lack of the faeces samples.
If the digestibility of the cell-wall constituents from the in vitro procedure is known, it is possible to calculate the percentage of digested cell-wall constituents; subtracting this value from digestibility in vivo provides the percentage of apparently digested cellular contents (% DCC). This percentage is the difference between the actually digested cellular contents and the bacterial excretion in the faeces. This % DCC can be correlated with the percentage of cellular contents (% CC), as is usually done for the digestible crude-protein content and the crude-protein content (both correlations have a similar physiological background).
The results are shown in Fig. 4, and the equations found are:

without legumes:

$$\% \text{ DCC} = 1.21 \% \text{ CC} - 29.0 \quad (n = 97;\ r^2 = 0.914;\ \text{RSD} = 2.67) \tag{13}$$

with legumes:

$$\% \text{ DCC} = 1.20 \% \text{ CC} - 28.7 \quad (n = 106;\ r^2 = 0.923;\ \text{RSD} = 2.63) \tag{14}$$

Both from the figure and from the equations it can be seen that the relationship between % DCC and % CC was very good. However, the lines deviate somewhat from the relationship found in the American samples of van Soest et al. (1965). They found: % DCC = 0.98 % CC — 13, in which 0.98 × 100 is considered to be the true digestibility of the cellular contents and 13 the bacterial excretion. In equation 14 a true digestibility of 120 is found while the bacterial excretion was 28.7 at zero

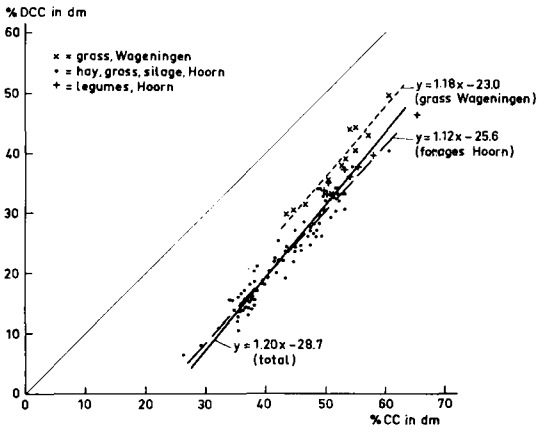

Fig. 4 Relationship between the percentage apparently digested cellular contents (% DCC) and the percentage cellular contents (% CC) of 106 Dutch forages

% CC. Of course, both coefficients are unrealistic and have to be discussed, therefore. In general it may be assumed that the bacterial excretion is proportionally related to the amount of substrate (= true digestibility) but it appears from these samples that this excretion is negatively related with the cellular contents (which itself is positively correlated with true digestibility). This is only possible if at higher percentages of cellular contents a larger part of these contents is transported so fast to the abomasum that it escapes from the microflora; this may explain the fact that the calculated true digestibility of the cellular contents is over 100. This decreasing bacterial excretion with increasing digestibility is also found by van Soest et al. in some cases (1966). However, at very low digestibilities and cellular contents, low bacterial excretion values may be expected again, because of the lack of substrate.

In these samples the bacterial excretion was about 20 units on an average, while it was about 13 units in van Soest's samples. However, the 12 grass samples from Wageningen showed this average difference of 13 units, while their separate regression line was off the general line. Consequently, omission of these 12 samples from the whole group reduced the RSD-value of the equations 11 and 12 from 2.6 to about 2.0. These separate regression equations are:

forages Hoorn:

$$\% \text{ DCC} = 1.12 \% \text{ CC} - 25.6 \quad (n = 94;\ r^2 = 0.944;\ \text{RSD} = 2.01) \tag{15}$$

grass Wageningen:

$$\% \text{ DCC} = 1.18 \% \text{ CC} - 23.0 \quad (n = 12;\ r^2 = 0.895;\ \text{RSD} = 2.03) \tag{16}$$

This bacterial excretion of about 13 units was found in digestion trials with cattle on some 20 hay samples from Wageningen as well, while in this group the 'summative equation' gave a RSD-value of as low as 1.7 (unpublished data). So it looks as if the bacterial excretion of the cattle and sheep in Wageningen was about 13 units in accordance with the data of van Soest, while the excretion in sheep at Hoorn was about 20 units. This might mean that either the rumen flora of the Hoorn sheep is not able to digest the food to the same extent as of the Wageningen sheep, or they are equally skilful but they are more efficient in metabolizing forage into bacterial bodies, thus reducing apparent digestibility. It might also imply that separate

equations should be used in different institutes depending on the composition of the microflora, which is just opposite to every contemporary philosophy.
However, in vitro apparent digestibility according to Tilley and Terry (1963) was determined in 17 Hoorn samples, in which it was found that digestibility in vivo and in vitro were almost equal. Thus the bacterial excretion was indeed about 20 units. Since all the determinations were done at Beltsville, with the same rumen fluid, these results suggest that the food itself strongly determines the bacterial excretion. If this is true, then it is not clear what caused the difference in bacterial excretion between the Wageningen and the Hoorn samples. For elucidating these differences, experiments are planned now in which vivo digestibility of some forages from Hoorn and Wageningen will be determined at both places.
Looking over all these results it is certain that, while we have some good in vitro methods for predicting forage digestibility, we do not know much about the way in which the food is actually digested. So it is obvious that still much detailed research has to be done for a clearer understanding of the digestion processes in ruminants.

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References

Deinum, B., 1966. Climate, nitrogen and grass. I. Research into the influence of light intensity, temperature, nitrogen and water supply on the production and chemical composition of grass. *Meded. LandbHogesch. Wageningen*, 66–11, pp. 91.

Deinum, B., Es, A. J. H. van & Soest, P. J. van, 1968. Climate, nitrogen and grass. II. The influence of light intensity, temperature and nitrogen on vivo digestibility of grass and the prediction of these effects from some chemical procedures. *Neth. J. agric. Sci.* 16: 217–223.

Hart, M. L. 't, 1945. Over de gehalten aan enkele minerale bestanddelen in gras. *Landbouwk. Tijdschr.* 57: 477–487.

Soest, P. J. van, 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fibre and lignin. *J. Ass. off. anal. Chem.* 46: 829–835.

Soest, P. J. van & Jones, L. H. P., 1967. The effect of silica upon digestibility decline. Paper P 102 at the ASDA meeting. Cornell University, Ithaca, N.Y.

Soest, P. J. van & Jones, L. H. P., 1968. Effect of silica in forages upon digestibility. *J. Dairy Sci.* 51: 1644–1648.

Soest, P. J. van & Moore, L. A., 1965. New chemical methods for analysis of forages for the purpose of predicting nutritive value. *Proc. 9th Int. Grassld Congr., Sao Paulo*: 785–789.

Soest, P. J. van & Wine, R. H., 1967. Use of detergent in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. *J. Ass. off. anal. Chem.* 50: 50–55.

Soest, P. J. van & Wine, R. H., 1968. The determination of lignin and cellulose in acid-detergent fibre with permanganate. *J. Ass. off. anal. Chem.* 51: 780–785.

Soest, P. J. van, Wine, R. H. & Moore, L. A., 1966. Estimation of true digestibility of forages by the in vitro digestion of cell walls. *Proc. 10th Int. Grassld Congr., Helsinki*: 438–441.

Tilley, J. M. A. & Terry, R. A., 1963. A two-stage technique for the in vitro digestion of forages. *J. Br. Grassld Soc.* 18: 104–111.