*Neth. J. agric. Sci. 19 (1971) 106—113*

# Prediction of forage digestibility from some laboratory procedures. 2. Comparison of in vivo digestibility at two institutes

B. Deinum

Department of Field Crops and Grassland Husbandry, Agricultural University, Wageningen, the Netherlands

Received: 1 March 1971

**Summary**

In the former paper some differences were noticed between the results of digestion trials at the institutes at Hoorn and Wageningen. In attempting to trace these differences a more detailed research was carried out on forages from both institutes.

It was found that the relationship between the in vivo and in vitro digestibility of organic matter did not significantly differ between the institutes. Likewise no difference was found in digestibility of the cell-wall constituents in relation to the lignin content, nor was there a significant difference in the relationship between the percentage of digestible-cellular contents and the percentage of cellular contents as far as forages of well managed pastures were concerned. However, 12 forages of highly digestible perennial ryegrass deviated from these farm forages at Wageningen, revealing a smaller excretion of bacterial and endogenous residue. Comparison of the two lignin procedures showed that 72% sulphuric-acid lignin gave rather consistent residual standard deviations of digestibility of cell-wall constituents, whereas the permanganate lignin gave smaller errors in the Wageningen forages, but greater errors in the Hoorn forages.

## Introduction

In an earlier paper on prediction of forage digestibility from the results of some laboratory procedure (Deinum and Van Soest, 1969), different regression lines were found for in vivo digestibility and some laboratory data on forages of the institutes at Hoorn and Wageningen. However it was not clear what could be the cause of these differences. To answer these questions more detailed research was done on another group of forages from both institutes and the results obtained are summarized in this publication.

## Experimental

Food and faeces samples of 35 forages of known in vivo apparent digestibility ($D_{vivo}$) were generously provided by the Institute for Livestock Feeding and Animal Research at Hoorn, the Netherlands. These forages (14 deep-frozen grasses, 10 hays, 8 silages and 3 legume pellets) were collected from permanent pastures of different farms and soils and some of them were herbage of different grass species grown for seed production. Likewise the Department of Animal Physiology, Agricultural University, Wageningen, the Netherlands, supplied similar samples of 16 forages of their own farm (1 dried grass, 11 hays and 4 silages) plus samples of the 12 forages of dried perennial ryegrass described already before (Deinum et al., 1968; Deinum and Van Soest, 1969). In vivo apparent digestibilities were obtained with sheep fed at about maintenance level.

All these samples were analysed for cell-wall constituents (%CWC, Van Soest and Wine, 1967), acid-detergent fibre (%ADF, Van Soest, 1963), 72% sulphuric-acid lignin (%SL, Van Soest, 1963) and permanganate lignin (%PL, Van Soest and Wine, 1968). In vitro true digestibility ($D_{vitro}$) of the forage samples was determined according to Van Soest et al. (1966) with the modification that the Erlenmeyer flasks were replaced by 250 $\times$ 25 mm test tubes, enabling 200 determinations in one run.

All constituents were determined as organic parts of dry matter, whereas the inorganic residues after CWC, ADF and $D_{vitro}$ determination were determined as well.

Analysing both forage and faeces samples enabled calculation of in vivo digestibility of all the constituents mentioned which may be more conclusive than in vitro digestibility.

## Results and discussion

*Comparison of $D_{vivo}$ and $D_{vitro}$*

The data presented in Fig. 1a and 1b show a very good agreement between in vivo and in vitro digestibility, except for two lawn grasses from Hoorn which also caused some deviations in the in vitro analyses at the Hoorn institute, and a hay from Wageningen given a high temperature treatment before cutting. For both institutes the relationship between the in vitro and in vivo digestibilities could be described with the same regression equation (all in vitro analyses were done in one run and the data were not corrected with the results of standards).

However the average difference between $D_{vitro}$ and $D_{vivo}$ was about 10 units in this case, whereas it was about 18 in the former publication. Two explanations are suggested for this discrepancy.

The first reason is that here digestibilities of organic matter are used, and in the earlier paper digestibilities of dry matter. $D_{vivo}$ of dry matter is always lower than $D_{vivo}$ of organic matter, because of low digestibility of the inorganic fraction. On the contrary $D_{vitro}$ of dry matter is always higher than $D_{vitro}$ of organic matter because the inorganic fraction of the residue, except soil particles, is dissolved in the neutral-detergent solution. Taken together this accounts for a difference of about 4 units.

The second reason is the rather low in vitro digestibility of the cell-wall constituents in the present material. Comparison of in vivo and in vitro digestibility of the cell-wall constituents showed that in this case the latter was about 5 units lower than the former whereas they were equal or even higher in the earlier paper. These lower in vitro digestibilities may be partly due to the replacement of Erlenmeyer flasks by test tubes (unpublished data and Van Soest et al., 1970) and by the incidently lower microbial activity in the week of analysis as found with the standard samples. These factors caused a discrepancy of about 3 units.

*Digestion of cell-wall constituents*

Digestibility of cell-wall constituents ($D_{CWC}$) depends on the composition of the cell walls and in this respect the percentage lignin of acid-detergent fibre (%SL/ADF or

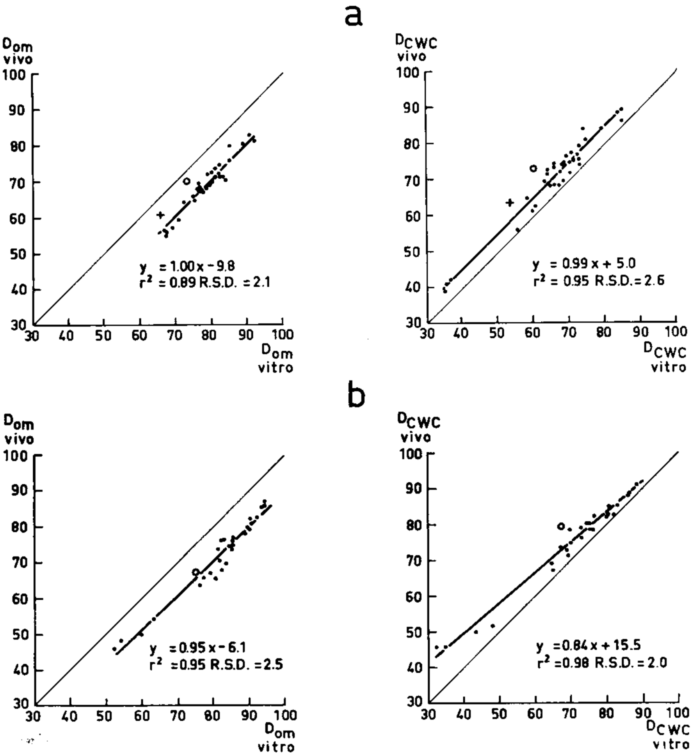


Fig. 1. Relationship between the digestibility of organic matter and cell-wall constituents in vivo and in vitro.
a. Hoorn: o *Poa pratensis* (Merion blue grass); + *Festuca ovina* var. *duriuscula*.
b. Wageningen: o high-temperature-treated hay.

%PL/ADF) is generally applied (Van Soest and Moore, 1965). In the former paper the relationship between $D_{CWC}$ and %PL/ADF showed rather large residual standard deviations (RSD) making detection of significant differences in $D_{CWC}$ between the institutes impossible. However, in their material Van Soest and Wine (1968) found a good correlation with %SL/ADF and a fairly good one with % PL/ADF. These considerations suggested a comparison of both methods on Dutch samples, and its description should precede a further discussion about the main subject.

*Comparison of lignin concentrations*

The data are summarized in Fig. 2a and 2b, in which it is shown that in the forage samples from Hoorn (Fig. 2a) the PL concentrations were always much higher than the SL concentrations in the forage samples without a good relationship between the two sets of data. This poor relationship was caused by the very deviating points of one lawn grass cut at heading stage and a silage with an improbably low SL percentage; omission of these points improved the relationship appreciably. These data suggest that as long as forages from pastures of good quality with a high percentage of favourable grass species are concerned Van Soest's system works satisfactory, although the corre-

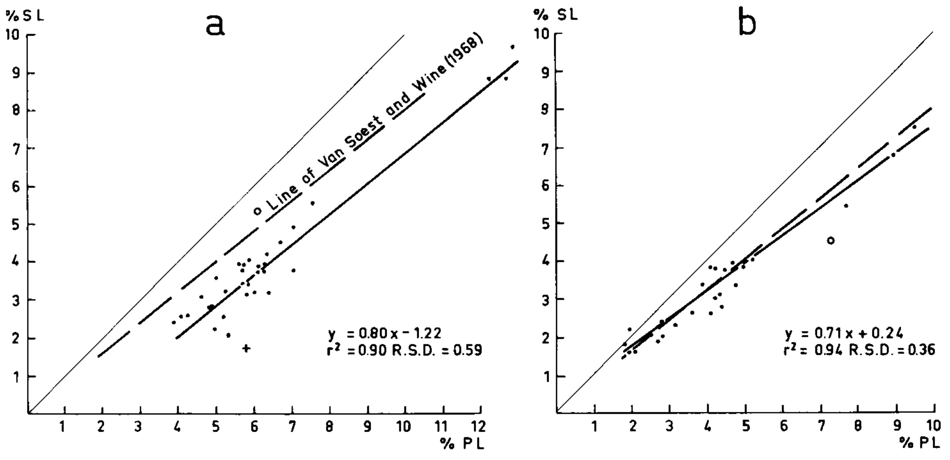


Fig. 2. Relationship between the concentration of 72% $H_2SO_4$ lignin (%SL) and $KMnO_4$ lignin (%PL) in dry matter.
a. Hoorn: o *Festuca rubra*; + grass silage.
b. Wageningen: o high-temperature-treated hay.

lation does not attain the height of Van Soest and Wine's (1968). In the faeces samples, however, on an average, both lignin contents were about the same with a fair relationship. Furthermore digestibility of SL (= $D_{SL}$) was distinctly negative except in two cases, whereas $D_{PL}$ was distinctly positive except in one forage.

Almost the same applies to the Wageningen samples (Fig. 2b), except for a better correlation between % SL and %PL in the forage samples, with a regression line almost identical to Van Soest and Wine's (1968). Furthermore the average value of $D_{SL}$ was about zero in these samples.

The practically equal contents of PL and SL in faeces might suggest that lignin in faeces is comparable to true lignin. If this is true, the negative values of $D_{SL}$ in the Hoorn samples may indicate that the 72% $H_2SO_4$ has dissolved part of the forage lignin, and therefore was too strong a solvent for lignin determinations in forages. However it remains obscure why $D_{SL}$ was negative in the Hoorn samples and about zero in the Wageningen samples. On the other hand the highly positive values of $D_{PL}$ suggest that $KMnO_4$ not only oxidizes lignin from ADF, but also part of the cellulose causing too high values, and therefore being too strong a solvent as well (in the SL procedure with 72% $H_2SO_4$, lignin is retained, whereas it is removed in the PL procedure with $KMnO_4$).

*$D_{CWC}$ in relation to cell-wall composition*

Digestibility of cell-wall constituents was related to the composition of the cell walls with single- or multiple-regression analysis; the results are summarized in Fig. 3 and Table 1. The most deviating points again were left out of consideration.

The results show that the Wageningen forages gave non-significantly differing regression equations from the Hoorn samples in the range tested, when %SL was considered. The residual standard deviations were approximately the same as in the former work, and higher than desirable. However, permanganate lignin gave much better results

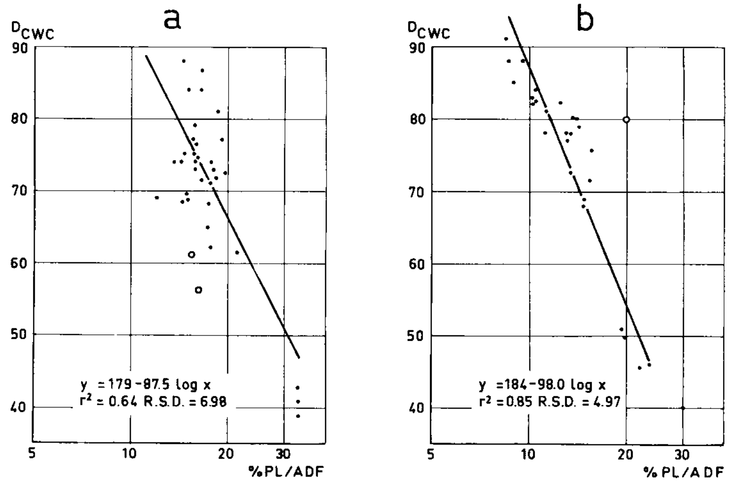


Fig. 3. Relationship between in vivo digestibility of the cell-wall constituents ($D_{CWC}$) and the permanganate lignin content of acid-detergent fibre (%PL/ADF).
a. Hoorn: o *Festuca rubra.*
b. Wageningen: o high-temperature-treated hay.

than sulphuric-acid lignin on the Wageningen forages, but inferior results on the Hoorn samples. The explanation for this is still to be found.

Furthermore is was immaterial whether lignin was expressed as %L/CWC, %L/ADF or as log (%L/ADF), although the middle one gave somewhat higher correlations. For better comparison with former work, however, log (%PL/ADF) is used in Fig. 3. Perhaps significant differences in way of expression might have been found when the correlations had been much higher.

Table 1. Relationship between in vivo digestibility of the cell-wall constituents ($D_{CWC}$) and the concentration of lignin and silica.

| | | $r^2$ | RSD |
|---|---|---|---|
| *Hoorn* | | | |
| $D_{CWC}$ | = 103.1 — 1.81% PL/ADF | 0.71 | 6.27 |
| | = 94.4 — 2.17% SL/ADF | 0.78 | 5.47 |
| | = 179.0 — 87.5 log (%PL/ADF) | 0.64 | 6.98 |
| | = 196.4 — 99.1 log (%PL/ADF) — 1.51 (%$SiO_2$/CWC) | 0.67 | 6.77 |
| | = 133.1 — 61.8 log (%SL/ADF) | 0.69 | 6.47 |
| | = 143.2 — 69.1 log (%SL/ADF) — 1.39 (%$SiO_2$/CWC) | 0.72 | 6.24 |
| %SL | = — 1.22 + 0.80% PL | 0.90 | 0.59 |
| *Wageningen* | | | |
| $D_{CWC}$ | = 116.4 — 3.07% PL/ADF | 0.91 | 3.87 |
| | = 112.7 — 3.60% SL/ADF | 0.70 | 7.07 |
| | = 184.7 — 98.0 log (%PL/ADF) | 0.85 | 4.97 |
| | = 187.6 — 102.3 log (%PL/ADF) + 0.77 (%$SiO_2$/CWC) | 0.86 | 4.92 |
| | = 164.6 — 89.0 log (%SL/ADF) | 0.65 | 7.66 |
| | = 165.6 — 90.1 log (%SL/ADF) + 0.16 (%$SiO_2$/CWC) | 0.65 | 7.82 |
| %SL | = 0.24 + 0.71% PL | 0.94 | 0.36 |

Lignin appeared to be the predominant factor determining digestibility. Including $SiO_2$ metabolized by the plant, measured as the difference between acid-detergent-insoluble and neutral-detergent-insoluble ash (Van Soest et al., 1970), did not improve the correlation in the Wageningen forages, but in the Hoorn samples a small, but significant increase of $r^2$ occurred. Whether this is caused by differences in soil or in grass species is not known.

Finally, it should be mentioned that the different regression equations should not correspond with those in earlier work, because in the present research all data were calculated as organic matter, which may be more meaningful than calculations in dry matter due to soil contamination in some samples.

*Digestion of cellular contents*

Knowing the in vivo apparent digestibility of organic matter and the concentration and in vivo digestibility of cell-wall constituents, the percentage of apparently digestible-cellular contents (%DCC) can be calculated and related to the percentage of cellular contents (%CC = 100 — %CWC). This is done in the same way as for comparing the percentage of digestible-crude protein with the percentage of crude protein in forages. The results are summarized in Fig. 4. The calculated regression lines (viz Fig. 4) show very good relationships in which the regression coefficients indicate the true digestibility of the cellular contents, and the intersections with the y axis the bacterial and endogenous excretion per 100 g organic-matter intake.

The Hoorn data show a true digestibility of the cellular contents of about 100% and a bacterial + endogenous excretion of about 13 units, which is almost equal to the findings of Van Soest (1967). The 16 forages from the farm of the Department of Animal Physiology at Wageningen are in close agreement with these data (Fig. 4b).

However, there is a significant difference between the 12 perennial ryegrass forages and the other 16 samples from Wageningen. These 12 perennial ryegrasses show a true digestibility of cellular contents of 111 which of course is unrealistic. Assuming a di-

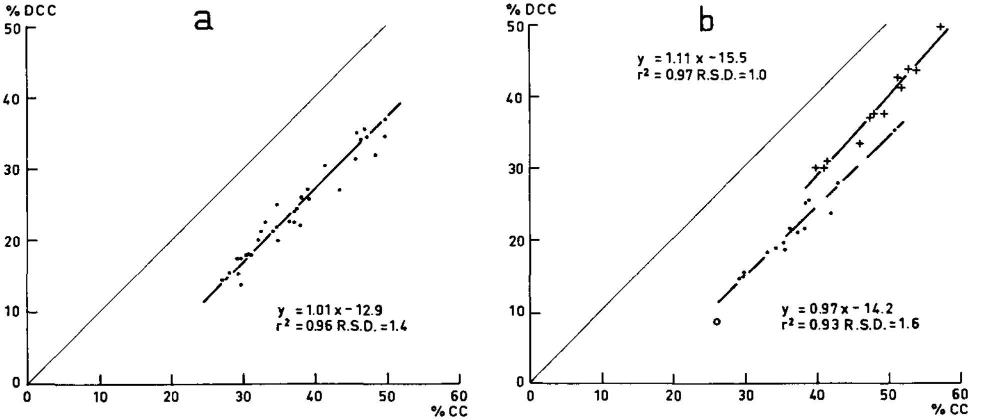


Fig. 4. Relationship between the percentage apparently digested-cellular contents (%DCC) and the percentage cellular contents (%CC) both as organic fractions of organic matter.
a. Hoorn.
b. Wageningen: o forages of Department of Animal Physiology; + perennial ryegrass forages.

gestibility of 100 makes the differences between %CC and %DCC equal to the bacterial and endogenous excretion and it appears that these values are lower in these 12 rye grasses than in the 16 other forages resulting in higher concentrations of apparently digestible cellular contents. These highly digestible perennial ryegrass forages have apparently caused different behaviour or metabolism of the rumen microflora. Their conversion of substrate into bacterial tissue may have been less efficient or the microflora formed is digested to a greater extend in the abomasum and small intestines, or both. This hypothesis is supported by the fact that % digestible-crude protein is higher in these 12 forages (viz Deinum et al., 1968) than calculated from the regression line of Dijkstra (1966). Moreover the regression coefficient of 1.11 on these 12 perennial ryegrasses suggests that bacterial plus endogenous excretion decreases with increasing digestibility which is also found by Van Soest et al. (1966) and Ellzey (1967) in cattle. However, the contrary was found by Kloppenstein and Woods (1967) in sheep.

The deviating results on these 12 perennial ryegrasses have led to the suggestion in the earlier paper (Deinum and Van Soest, 1969) that the Hoorn and Wageningen digestion trials were not comparable, but these new findings show only minor differences between the trials of the two institutes and the 12 highly digestible ryegrasses have been responsable for the discrepancy.

Meanwhile it should be noticed that the bacterial and endogenous excretion was about 20 units in the earlier paper, whereas it is about 13 units in this one. Again it can be calculated that this is mainly caused by the way of expressing the results: organic compounds in percentage of organic matter versus dry-matter compounds in percentage of dry matter.

Summarizing these data it is clear that the digestion trials at Hoorn and Wageningen give comparable results, but that within an institute some forages may be digested in another way by the rumen micro organisms than would be expected from preceding experiments.

## Acknowledgment

The generous provision of forage and faeces samples by Dr N. D. Dijkstra, Institute for Livestock Feeding and Animal Research, Hoorn, and by Dr A. J. H. van Es, Department of Animal Physiology, Agricultural University, Wageningen, is gratefully appreciated.

The author is much indebted to Mrs Tyne van der Ven and Mrs Evelien Zantman for their great share in the chemical analyses.

## References

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

Deinum, B. & P. J. Van Soest, 1969. Prediction of forage digestibility from some laboratory procedures. *Neth. J. agric. Sci.* 17: 119–127.

Dijkstra, N. D., 1966. Estimation of the nutritive value of fresh roughage. *Proc. 10th Int. Grassld Congr.* (Helsinki) 393–397.

Ellzey, H. D., 1967. Report to NC-64 meeting on 'The development and application of laboratory methods for determining forage quality'. Mimeographed, October 1967. Ames, Iowa.

Kloppenstein, T. & W. Woods, 1967. Report to NC-64 meeting on 'The development and application of laboratory methods for determining forage quality'. Mimeographed, October 1967. Ames, Iowa.

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

Van Soest, P. J., 1967. Development of a comprehensive system of feed analysis and its application to forages. *J. anim. Sci.* 26: 119–128.

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

Van Soest, P. J., K. D. Sayre & F. E. Lovelace, 1970. Progress of work and principal accomplishments. New York contributing Project to NC-64 'Laboratory methods for estimating forage quality'. Mimeographed, pp. 10.

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

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

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