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# Digestibility of cell walls of ageing grass leaves as estimated from *in vitro* and gas production techniques

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## Summary

The utilization of roughages is largely dependent on the digestion of cell walls (CW). The dynamics of digestion of CW of a leaf of *Lolium multiflorum* at different stages of maturity were quantified. CW digestibility was estimated from *in vitro* and gas production techniques. Ageing of the leaf resulted in a slightly lower rate of *in vitro* gas production from CW fermentation. The rate constant was reduced and the half time increased. Total gas production per gram CW digested after 120 hours increased with age of the leaf. CW digestibility after 48 hours *in vitro* was underestimated when calculated from gas production measurements. The changes in gas production parameters with increasing CW maturity and underestimation of digestibility from gas production were probably caused by the increasing resistance of CW to degradation and by alterations in the contribution of different fermentation processes to CW degradation.

*Keywords:* cell walls, digestion kinetics, fermentative gas production, *in vitro* digestibility, italian ryegrass, *Lolium multiflorum*.

## Introduction

Recent changes in intensive grassland management practices toward lower fertilizer inputs have caused a shift of interest from increasing production to improving feeding quality. Therefore, a better understanding is required of the characteristics of CW in degradation processes during rumen digestion, since CW are the least degradable part of roughages. In a glasshouse experiment the CW digestion characteristics of plant organs were related to morphological and physiological features of initiation, ageing and senescence.

CW primarily consist of structural polysaccharides, which are fermented by rumen microbes, to produce microbial matter, volatile fatty acids and gasses ($CO_2$ and $CH_4$). The rate and extent of digestion can be estimated from the disappearance of substrate

or the formation of fermentation end-products *in vitro*. In this paper the *in vitro* digestion method according to Goering & Van Soest (1970) was compared with the cumulative gas production technique described by Theodorou *et al.* (in press).

## Materials and methods

Italian ryegrass (*Lolium multiflorum*) was sown in August 1992, in a glasshouse at 18/13°C day/night temperature. When the ligule of the fourth leaf on the main tiller was visible, the plants were cut 2 cm above ground level. Plants remained vegetative. Leaves from insertion level 7 on the main tiller were harvested when first fully expanded (ligule visible) and at 14, 35 and 49 days thereafter. Samples were oven dried (70°C), weighed and ground. Cell wall digestibility ($D_{48vitro}$) was determined using the method described by Goering & Van Soest (1970).

Isolated CW (neutral detergent and protease treated) were incubated with rumen fluid for 120 hours and gas production was recorded, according to Theodorou *et al.* (in press). The resulting cumulative gas production curve was fitted using a sigmoidal 'switch-on' response curve:

$$Y_t = \frac{Y_\infty (t-L)^c}{(H-L)^c + (t-L)^c}$$

In this equation, $Y_t$ denotes the yield of gas at time t (hours) after incubation per g digested CW after 120 hours (ml.g $DCW^{-1}$); $Y_\infty$ represents the asymptotic gas production (ml.g $DCW^{-1}$). L is the lag phase (hours); H is the time after incubation at which half of the final amount of gas has been formed (hours) and c is a constant determining the sharpness of the switching characteristic. After the gas production measurement, the proportion of CW digested was determined ($D_{120}$). From $D_{120}$ and the ratio between $Y_{48}$ and $Y_{120}$, the CW digestion after 48 hours was calculated ($D_{48gas}$).

## Results

In Table 1 the parameters of gas production and estimates of cell wall digestibility are presented. Maturation of leaf 7 resulted in a decline in the rate of gas production. The value of c decreased with subsequent harvests, and H and L increased. Also $Y_{120}$ and $Y_\infty$ increased. The effects of maturation are shown in Figure 1.

The digestibility of CW after 48 and 120 hours decreased with ageing of the leaf. The calculated $D_{48gas}$ resulted in lower estimates of CW digestibility than $D_{48vitro}$. This discrepancy tended to increase with ageing.

*Table 1. Parameters of gas production from isolated cell walls of leaf 7 at different dates after appearance of the ligule, with standard errors of means (SEM). Significant differences (Tukey, $\alpha$=0.05) are indicated with superscripts.*

| Parameter | Days after appearance of the ligule | | | | SEM |
|---|---|---|---|---|---|
| | 0 | 14 | 35 | 49 | |
| $Y_{120}$ (ml/g DCW) | $360^{a}$ | $358^{a}$ | $368^{b}$ | $364^{ab}$ | 2.72 |
| $Y_{\infty}$ (ml/g DCW) | $366^{a}$ | $366^{a}$ | $386^{b}$ | $385^{b}$ | 1.73 |
| H (hours) | $19.0^{a}$ | $20.6^{b}$ | $23.4^{c}$ | $22.9^{c}$ | 0.32 |
| L (hours) | $6.7^{a}$ | $8.6^{b}$ | $10.2^{c}$ | $9.8^{c}$ | 0.41 |
| c (-) | $1.80^{a}$ | $1.57^{b}$ | $1.38^{c}$ | $1.36^{c}$ | 0.07 |
| $D_{120}$ (%) | $90.9^{a}$ | $88.5^{b}$ | $85.7^{c}$ | $85.2^{c}$ | 0.37 |
| $D_{48vitro}$ (%) | $84.9^{a}$ | $80.8^{b}$ | $79.6^{b}$ | $78.1^{b}$ | 1.24 |
| $D_{48gas}$ (%) | $82.9^{a}$ | $78.4^{b}$ | $72.8^{c}$ | $73.2^{c}$ | 0.90 |

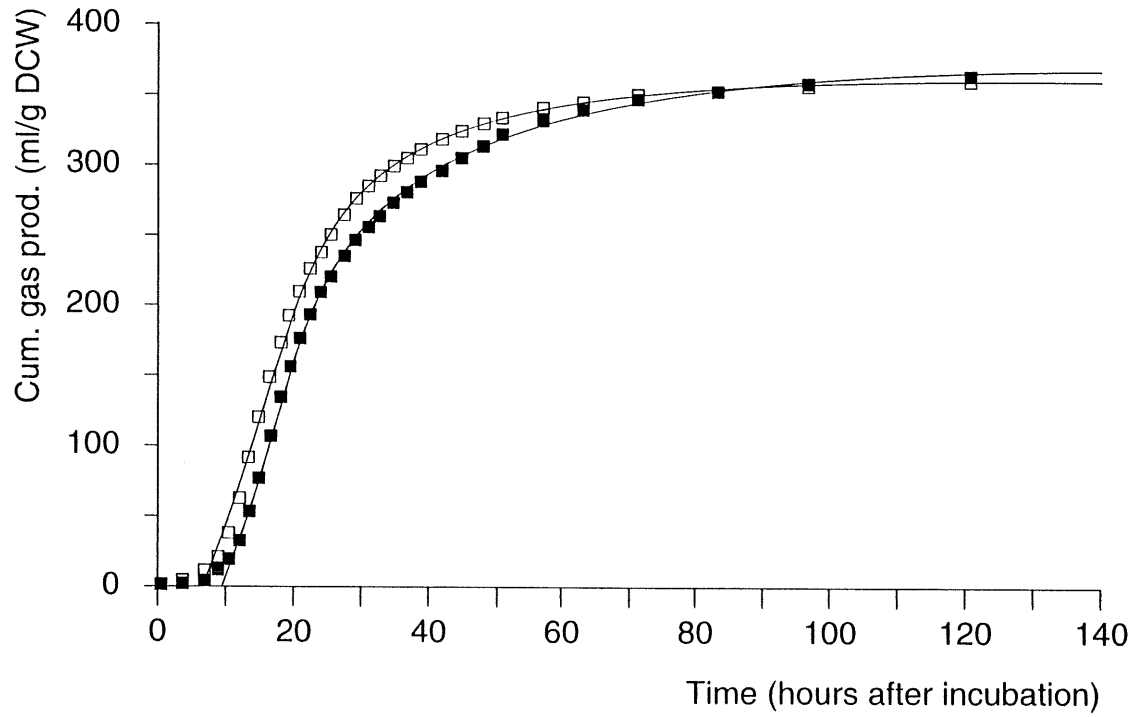


*Figure 1. Cumulative gas production of isolated cell walls of leaf 7 in a young* ( □ ) and mature ( ■ ) *stage (respectively 0 and 49 days after appearance). Symbols and lines represent measured values and fitted curves, respectively.*

## Discussion

The rate and extent of digestion were reduced with ageing of the leaf. This was probably caused by changes in CW properties (Fry, 1988), which increased the resistance of CW to microbial breakdown. The changes in CW characteristics are probably not due to changes in chemical composition, but to an increase in the interpolymeric bonding (Fry, 1988). The rate of decline in digestibility during ageing was low, which is usually observed in such leaves of higher insertion level (Nordang *et al.*, 1992).

Several pathways of microbial fermentation occur in the rumen. Per g DCW, fermentation end-products are produced in different amounts and proportions (Van Houtert, 1993). The final gas production g $DCW^{-1}$ gives an indication of the contribution of the various fermentation pathways to CW degradation. When CW degradability declines, fermentation shifts to a pathway yielding more gas g $DCW^{-1}$ (Beuvink & Spoelstra, 1992). This can explain the higher final gas production g $DCW^{-1}$ with increasing maturity, observed in this experiment.

Shifts between pathways may occur in the course of the incubation period. Gas production from easily degradable substrate fermented in early stages of fermentation will be low relative to gas production per g DCW between 48 and 120 hours after incubation. Additionally, when substrate availability decreases, turnover of microbial matter after lysis might occur, resulting in extra gas production. Consequently, $D_{48gas}$ underestimated CW digestibility. These phenomena are enhanced when CW degradability declines. Therefore, the difference between $D_{48vitro}$ and $D_{48gas}$ increased with ageing.

The cumulative gas production technique might prove a usefull technique for assessing the degradation of roughages and the resulting formation of fermentation products. However, the relation between substrate disappearance and production of volatile fatty acids, microbial matter and gasses during fermentation has to be established.

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