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This Week's Citation Classic

Tilley J M A & Terry R A. A two-stage technique for the *in vitro* digestion of forage crops. *J. Brit. Grassland Soc.* **18**:104-11, 1963.

A laboratory technique for determining the digestibility of dried forages is described. It involves incubation first with rumen liquor and then with acid pepsin solution. Using 146 herbage samples of known *in vivo* digestibility (Y) the regression equation Y = 0.99X - 1.01 (SE± 2.31) was calculated, where X = *in vitro* digestibility. [The *SCI*® indicates that this paper has been cited over 645 times since 1963.]

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"By the late 1950s, our colleagues at this Institute had measured the *in vivo* digestibility by sheep of many different herbages, and had shown that digestibility could be an important index of the relative feeding value of a herbage.[1] However, such measurements were difficult, costly, and time-consuming when carried out with animals; a technique for the prediction of digestibility by a laboratory method was obviously desirable.

"Chemical analysis of herbages was known not to be adequate for such prediction purposes. Therefore, we looked for a biological method in which, under conditions which simulated those within the rumen of a sheep (anaerobic, near neutral pH, blood heat), small samples of herbage could be digested with crude rumen liquor rich in microorganisms.

" 'Bubbles,' a tame sheep with a permanent rumen fistula, provided the necessary liquor. Over some 10 years, he willingly gave to science every Monday morning most of what was left over from his Sunday supper. However, even with Bubbles' cooperation, we did not at first obtain the good correspondence between *in vivo* and *in vitro* digestibility for which we had hoped. We eventually realised that results were especially poor with young leafy herbages of high protein content and that rumen inocula, though capable of dealing with the digestible structural carbohydrates, was relatively ineffective for the digestion of the protein in our heat-dried samples. Digestible protein could, however, be readily removed by a second-stage treatment with acid-pepsin. Following this, *in vitro* and *in vivo* results were in very close agreement over a very wide range of herbage samples.

"Between the publication of our initial results in 1961[2] and this detailed paper in 1963, considerable experience with the method had been gained by ourselves and by workers in other laboratories. In writing this paper, we therefore set out to provide a practical guide to a new technique for herbage evaluation in a simple and clear form. We are pleased if this paper has served to stimulate interest in the biological approach to herbage analysis. We are also pleased that, although less crude (and less smelly). methods based on the use of enzymes rather than rumen liquor have subsequently been proposed,[3] none gives such a close identity with *in vivo* digestibility as does our relatively crude technique which has stood the test of time. It is probably for the foregoing reasons, and because the technique described is appropriate for use throughout the world, that this publication has been so frequently cited."

1. **Minson D J, Raymond W F & Harris C E.** Studies in the digestibility of herbage. VIII. The digestibility of S37 cocksfoot. S23 ryegrass and S24 ryegrass. *J. Brit. Grassland Soc.* **15**: 174-80, 1960.
2. **Tilley J M A, Deriaz R E** & **Terry R A.** The *in vitro* measurement of herbage digestibility and assessment of nutritive value. *Proceedings of the 8th International Grassland Congress, Reading, 1960.* p. 533-7.
3. **Jones D I H & Hayward V M.** The effect of pepsin pretreatment of herbage on the prediction of dry matter digestibility from solubility in fungal cellulase solutions. *J. Sci. Food Agr.* **26**:711-8, 1975.