Available online at www.sciencedirect.com
SCIENCE DIRECT®
Animal Feed Science and Technology
123–124 (2005) 501–515
ANIMAL FEED SCIENCE AND TECHNOLOGY
www.elsevier.com/locate/anifeedsci

Alternative methodologies – stretching the in vitro box

F.L. Mould *, K.E. Kliem, R. Morgan

School of Agriculture, Policy and Development, University of Reading, Earley Gate, PO Box 237, Reading RG6 6AR, UK

Abstract

Current gas-based in vitro evaluation systems are extremely powerful research techniques. However they have the potential to generate a great deal more than simple fermentation dynamics. Details from four experiments are presented in which adaptation, and novel application, of an in vitro system allowed widely differing objectives to be examined. In the first two studies, complement methodologies were utilised. In such assays, an activity or outcome is inferred through the occurrence of a secondary event rather than by direct observation. Using an N-deficient incubation medium, the increase in starch fermentation, when supplemented with individual amino acids (i.e., known level of N) relative to that of urea (i.e., known quantity and N availability), provided an estimate of their microbial utilisation. Due to the low level of response observed with some amino acids (notably methionine and lysine), it was concluded, that they may not need to be offered in a rumen-inert form to escape rumen microbial degradation. In another experiment, the extent to which degradation of plant cell wall components was inhibited by lipid supplementation was evaluated using fermentation gas release profiles of washed hay. The different responses due to lipid source and level of inclusion suggested that the degree of rumen protection required to ameliorate this depression was supplement dependent.

That in vitro inocula differ in their microbial composition is of little interest per se, as long as the outcome is the same (i.e., that similar substrates are degraded at comparable rates and end-product release is equivalent). However where a microbial population is deficient in a particular activity, increasing the level of inoculation will have no benefit. Estimates of hydrolytic activity were obtained by examining fermentation kinetics of specific substrates. A number of studies identified a fundamental

Abbreviations: DM, dry matter; iOMD, in vitro organic matter degradation; IVTDMD, in vitro true dry matter digestion; ME, metabolizable energy; NDF, neutral detergent fibre; NWNS, non-soluble non-structural carbohydrate; OM, organic matter; RPT, Reading Pressure Technique; VFA, volatile fatty acids; WH, washed hay; WSC, water soluble carbohydrate

* Corresponding author.
E-mail address: f.mould@rdg.ac.uk (F.L. Mould).

0377-8401/$ – see front matter
doi:10.1016/j.anifeedsci.2005.04.023

difference between rumen fluid and faecal inocula, with the latter having a lower fibrolytic activity, which could not be completely attributed to microbial numbers. The majority of forage maize is offered as an ensiled feed, however most of the information on which decisions such as choice of variety, crop management and harvesting date are made is based on fresh crop measurements. As such, an attempt was made to estimate ensiled maize quality from an in vitro analysis of the fresh crop. Fermentation profiles and chemical analysis confirmed changes in crop composition over the growing season, and loss of labile carbohydrates during ensiling. In addition, examination of degradation residues allowed metabolizable energy (ME) contents to be estimated. Due to difficulties associated with starch analysis, the observation that this parameter could be predicted by difference (together with an assumed degradability), allowed an estimate of ensiled maize ME to be developed from fresh material. In addition, the contribution of the main carbohydrates towards ME showed the importance of delaying harvest until maximum starch content has been achieved.

Keywords: Complement methodology; Amino acid utilisation; Fibre degradation; Hydrolytic activity; Maize silage quality prediction

1. Introduction

The two-stage methodology of Tilley and Terry (1963), which provides an in vitro estimate of in vivo digestibility, can be considered one of the great advances in ruminant feedstuff evaluation. Modifications by Goering and Van Soest (1970) led to the IVTDMD (in vitro true dry matter (DM) digestibility) assay that is still widely used. However, as the extent of rumen degradation of a feedstuff is the product of ruminal residence time and rate of degradation, these methodologies are deficient in that they provide no kinetic information on degradation. It is, therefore, possible for two feeds to have similar end-point degradation values, but different rates of degradation. This is of fundamental importance, as feeds with higher degradation rates tend to be consumed in greater quantity. In addition, as these assays are generally made after an extended incubation period (usually 48–96 h), it is possible that degradation is over-estimated and subtle differences among similar feeds lost.

While gas production systems (e.g., Menke et al., 1979) allow fermentation gas release profiles to be used to estimate degradation kinetics, the relationship between these is highly substrate dependent. Thus, use of gas values alone provides insufficient information to assess widely differing feedstuffs. Current systems (e.g., Pell and Schofield, 1993; Davies et al., 1995; Cone et al., 1996; Mauricio et al., 1999) vary in their complexity, capacity and assay capabilities. However they have the potential to provide a great deal more information than an estimate of end-point degradation. For instance, Colombatto et al. (2003) examined the influence of fibrolytic enzymes on hydrolysis and fermentation of the pure carbohydrate substrates cellulose and xylan by mixed ruminal microorganisms using in vitro gas release kinetics. Similarly, Mlambo et al. (2002) assessed the effectiveness of alkalis to inactivate tannins in leguminous tree fruits, and so improve their nutritive value in Zimbabwean smallholder goat systems, by measuring changes in gas release.

The following case studies briefly detail novel applications of the Reading Pressure Technique (RPT; Mauricio et al., 1999) modified for specific situations.