

## The effect of rumen fluid storage time on digestive capacity with five forage/browse samples

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### Abstract

Rumen fluid collected for *in vitro* digestion studies from wild herbivores cannot always be used immediately and must be stored. This experiment examined the effect of storage time on the digestive ability of sheep rumen fluid using five feeds of differing digestibility (three browse species, a temperate grass and a tropical grass).

Rumen fluid was collected, chilled to 18°C and stored under CO<sub>2</sub> for 24h and 48h before being used at the same time as fresh rumen fluid. A 72h rumen fluid/buffer digestion and a 24h acid pepsin digestion was used, to determine *in vitro* digestibility. Three separate runs were made, with triplicate samples of each feed in each run.

Overall there was a slight decline in digestibility: 50.9, 50.2 and 49.0% with storage times of 0h, 24h and 48h, but differences were not significant ( $P>0.05$ ). There was no feed  $\times$  storage time interaction ( $P>0.05$ ). It is proposed that storage for up to 48h at 18°C would be acceptable for examining digestive capacity of rumen fluid collected from wild animals in remote locations.

### Introduction

For assessment of *in vitro* digestion of plant samples, it is recommended that the rumen fluid collected from donor animals be maintained at

about 39°C and used as soon as possible after collection (Tilley and Terry 1963).

Under some situations this ideal would be impossible to achieve. Such a situation arose in connection with a project to assess the capacity of rumen fluid from wild browsers to digest tanniferous plant species. It was considered impractical to move the facilities from a well established *in vitro* laboratory to the field sites where game were shot. Since the samples could not be transported to the laboratory within a short time, we needed to know the effect of storage time on the digestive capacity of rumen fluid. This study sought to obtain data on the effects of storage time of rumen fluid on digestive capacity.

### Materials and methods

#### Plant samples

Five freeze-dried plant samples of differing digestibility were used. These were:

1. *Acacia karoo* — tropical tree/browse
2. *Combretum apiculatum* — tropical tree/browse
3. *Desmanthus virgatus* — tropical shrub/browse
4. *Lolium multiflorum* — temperate grass
5. *Panicum maximum* — tropical grass (Standard)

The plant samples were milled to pass a 1mm sieve and stored in bottles for use.

#### Rumen fluid samples

Rumen fluid was obtained from 2 rumen-fistulated merino wethers that had been fed on an average quality lucerne (*Medicago sativa*) hay for 2 months. Rumen fluid, strained through 1 layer of cheese cloth, was collected in conical flasks. Anaerobic conditions were maintained by slowly bubbling CO<sub>2</sub> through the flasks whilst chilling on iced water to 18°C. Thereafter, the flasks were held in a cooler box at 18°C. Samples

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were taken so that, on the one day, samples stored for 48h, 24h and 0h were available for each *in vitro* run. The rumen fluid was re-warmed to 39°C in a waterbath, while slowly bubbling CO<sub>2</sub> through the flasks. The rate of warming was limited to 5°C every 30 minutes.

#### *In vitro* technique

A modified Tilley and Terry (1963) method was used. The modifications consisted of adding 5ml of a urea solution (8.6g/l) to each plant sample in the tubes before adding the 50ml of rumen fluid/buffer medium. In addition, the rumen fluid/buffer incubation was increased from 48h to 72h (Drew 1966), and the acid pepsin stage was correspondingly reduced from 48h to 24h. Appropriate blank tubes were also used. Tube contents were mixed by shaking 3 times/day. At the end of the 72h digestion, the tubes were centrifuged for 20min at 1800g, the supernatant removed by suction tube, 50ml of acid pepsin added and the tube contents mixed and incubated. At the end of 24h, tubes were centrifuged for 10min at 1800g, the supernatant removed by suction tube, 60ml of hot (90°C) water added, the tubes thoroughly mixed on a magnetic stirrer, centrifuged once more for 10min at 1800g, supernatant removed and the residue dried in the digestion tubes.

Dry matter and ash were measured on duplicate samples of each feed, and also on the residues. The *in vitro* organic matter digestion (IVOMD) was calculated for each sample in the normal manner.

#### Statistical analysis

There were  $6 \times 3 \times 3 = 54$  tubes in each of three runs, *i.e.* 5 feeds and a blank  $\times$  3 rumen fluid treatments (3 storage times)  $\times$  3 samples per feed. In each run, triplicate samples were used to obtain a mean IVOMD for each plant material. The 3 separate runs were treated as replicates in a  $5 \times 3$  randomised design.

#### Results

In each run, the digestibility of the 5 feeds ranked in the same order, namely: *Lolium multiflorum* > *Panicum maximum* > *Desmanthus virgatus* > *Combretum apiculatum* > *Acacia karoo*. Overall,

the species differences were highly significant ( $P < 0.001$ ) (Table 1). As a source of variation, feeds accounted for 98% of the total.

**Table 1.** Effect of storage time of rumen fluid (RF) on the digestion of 5 feeds. Means from 3 replicate runs, each with triplicate samples of the feeds.

Feed	OM digestibility (g/100g) with RF stored for:			Mean
	0h	24h	48h	
<i>Acacia karoo</i>	31.1	30.5	28.8	30.1e
<i>Combretum apiculatum</i>	33.7	35.8	36.0	35.2d
<i>Desmanthus virgatus</i>	47.4	45.9	44.2	45.8c
<i>Lolium multiflorum</i>	82.6	81.8	79.8	81.4a
<i>Panicum maximum</i>	59.8	57.1	56.0	57.6b
Mean	50.9a <sup>1</sup>	50.2a	49.0a	50.0

<sup>1</sup> Within rows or columns, means followed by the same letters do not differ significantly ( $P > 0.05$ ).

Overall mean digestibility values declined slightly with length of storage but did not differ significantly ( $P > 0.05$ ). The mean values were: 50.9, 50.2 and 49.0 g/100 g for the 0, 24 and 48h storage times respectively. Also there was no feed  $\times$  rumen fluid treatment interaction ( $P > 0.05$ ).

#### Discussion

The results clearly show that, with sheep rumen fluid, storage of the chilled rumen fluid for up to 48h resulted in no significant depression in digestive capacity of the rumen fluid with the range of feeds examined. It should be noted, however, that we used a 72h digestion in rumen fluid/buffer. Results with only 48h digestion could be different. The longer digestion time tends to overcome varying lag-phases which can occur with tanniferous feeds and stored rumen fluid (R.J. Jones, unpublished data).

The use of 18°C as the temperature for storage met no specific objective. This treatment could be repeated readily under field conditions remote from the laboratory. It was chosen: to suppress microbial activity and hence CO<sub>2</sub> production so that rumen fluid could be sealed in vacuum flasks without danger of the screw tops blowing off; to minimise any shock to rumen micro-organisms from excessive chilling and subsequent re-warming; and to minimise the time to reactivate the rumen fluid when warmed to 39°C prior to adding to the digestion tubes.

For field sampling with wild animals, we make the assumption that results will be the same as for

the sheep. We have no way to verify this since we had no access to fistulated giraffe or kudu (the target species) running under range conditions and which could be sampled sequentially. The assumption, however, seems reasonable in the light of our unpublished results indicating that rumen fluid from giraffe under *in vitro* conditions ranked a range of feeds in the same order as did rumen fluid from sheep in the same digestion batch.

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