The influence of inoculum source on *in vitro* digestibility

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A series of *in vitro* incubations were performed in which a constant substrate (antelope cube) was used and only the inoculum source was varied. With an inoculum obtained from 7 different wild ruminants, gas production rate varied considerably and was influenced by season. This was further investigated by testing fermentation rate on the same substrate with rumen fluid obtained from free-ranging kudu on a monthly basis over the year. It was found that fermentative ability declined during the winter. Thus *in vitro* digestibility of a given substrate is a function of inoculum source, time of the year and feed selection by the donor animal.

'n Reeks van *in vitro* inkubasies is gedoen waartydens een substraat (wildsblok) gebruik is, en slegs die bron van inokulum verskil het. Met 'n inokulum wat van 7 verskillende herkouers verkry is, het die tempo van gasproduksie beduidend verskil en is deur die seisoen beïnvloed. Dit is verder ondersoek deur die tempo van fermentasie op dieselfde substraat te toets met rumenvloeistof wat verkry is van vryweiende koedoes op 'n maandelikse grondslag vir 'n jaar. Daar is gevind dat die gistingsvermoë afgeneem het tydens die winter. Die *in vitro* verteerbaarheid van 'n gegewe substraat is dus 'n funksie van die bron van inokulum, tyd van die jaar en voedselkeuse van die skenkerdier.

Keywords: *in vitro* digestibility, inoculum source, fermentation rate, wild ruminants, season, feed preference of donor

Introduction

The digestibility of plants consumed by wild herbivores is often determined using the Tilley and Terry (1963) method, with domestic stock on a constant ration as rumen fluid donors. This could lead to over- or under estimation of actual substrate digestibility by a particular species utilizing the plant or parts thereof.

Fermentation rate is generally expressed as the volume or moles of gas produced per hour per unit mass dry matter. The relationship between gas production and VFA has also often been used with a correction factor to estimate VFA production. Fermentation rate *in vitro* depends on various factors such as substrate composition, inoculum volume, inoculum pH, buffer composition and amount of substrate. When these factors are kept constant a direct relationship should emerge between *in vitro* dry matter disappearance (IVDMD) and gas produced over time.

A series of *in vitro* incubations were undertaken in which only the inoculum source was varied in order to illustrate the effect of this on *in vitro* digestibility in terms of gas production. In a second set of incubations done monthly for a year using kudu as the inoculum donor, the influence of season on the fermentative capacity of the rumen fluid is illustrated.

Methods

The incubations were performed in a waterbath at 39°C in 30 ml glass vials fitted with rubber stoppers. Each contained 15 ml buffer (adjusted to pH 6,5), 1 g substrate and 5 ml rumen fluid. The substrate used throughout was a commercial antelope cube, dried and milled to 2 mm with a composition of 18,6% CP, 14,3% CF, 6,9% ash, 22,5% NDF, 6,5% hemi-cellulose, 12,4% cellulose and 4,6% ADL.

The various ruminant species used in April and October as inoculum donors are listed in Figures 1 and 2. The animals were shot while in their natural habitat. Rumen fluid samples were obtained within 20 minutes after death and squeezed through a double layer of cheesecloth. The strained fluid was bubbled with CO_2 to maintain anaerobic conditions. Kudu from the Nylsvley Reserve were used in the same way on a monthly basis, to monitor variations in rumen fluid vitality as influenced by changes in their natural browse through the year.

The incubations lasted for 20 hours after which time the DM digested was calculated. The gas produced during the 20 h was measured on a continuous basis and it is assumed that the rate of DM disappearance is described by the line illustrating the rate of gas production. Fermentation of endogenous reserves of glycogen in the protozoa as well as small particles of plant material in the inoculum did take place since gas was produced to a limited degree in the controls which contained no substrate. These were subtracted from the incubations with substrate.

Results

The rates of digestion during April (Figure 1), when feeding conditions were favourable, and during October before the first rains (Figure 2) are presented. A wider variation exists in the potential of the various types of rumen fluid to digest the substate in April as compared to October. The general trend is a lowering of the rumen fluid's ability to digest the same substrate in the dry season. In the gemsbok this decline was 25% and in the eland 20% while the blue wildebeest showed a 26% reduction in fermentation. In the kudu this reduction was very small, while the springbok and red hartebeest showed an 11% and 33% increase.

The magnitude of this trend was investigated in kudu on the Nylsvley Nature Reserve. *In vitro* digestibility of the same substrate with rumen fluid obtained from free ranging kudu was tested on a monthly basis. All other conditions remained constant. In Figure 3 it is again apparent that the fermentative ability of the rumen fluid declined during the winter.

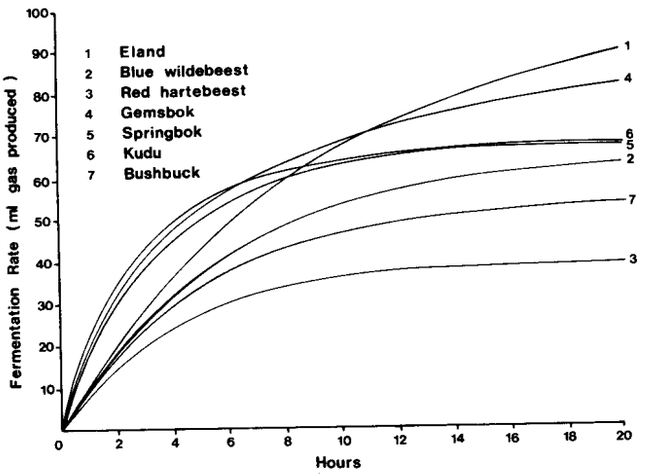

Figure 1 Fermentation rate of the same substrate *in vitro*, measured in terms of gas production using 7 different wild ruminants as inoculum source during April with favourable feeding conditions.

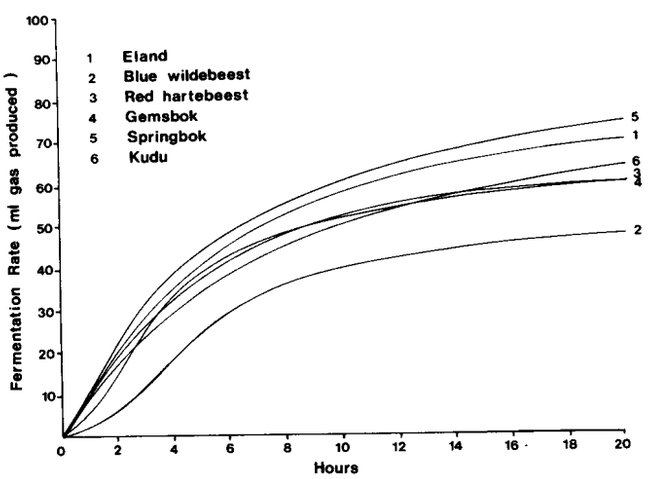

Figure 2 Fermentation rate of the same substrate *in vitro*, measured in terms of gas production using 6 different wild ruminants as inoculum source during October with unfavourable feeding conditions.

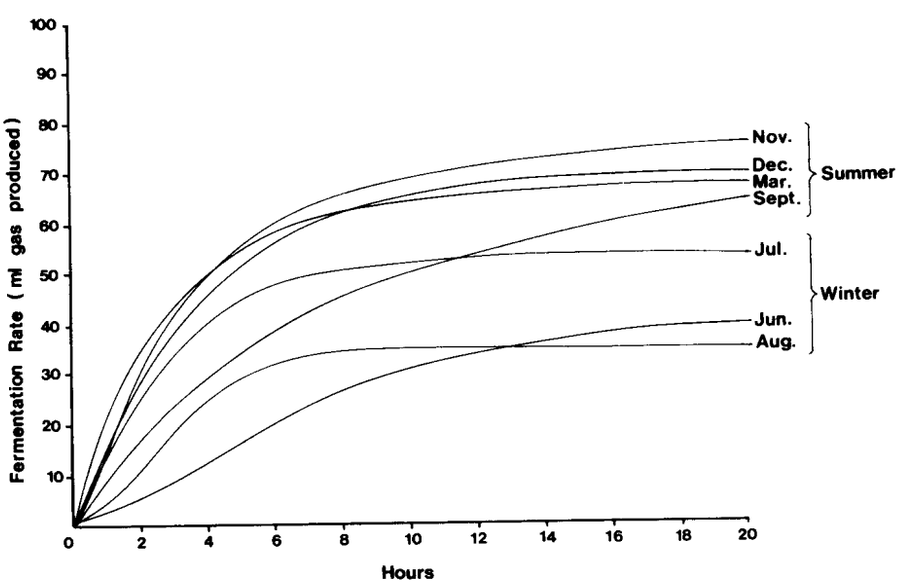

Figure 3 Fermentation rate of the same substrate *in vitro*, measured in terms of gas production using kudu from Nylsvley Nature Reserve as inoculum source at various times of the year.

Discussion

The microbial population in the rumen is dynamic and has the potential to adapt towards optimal utilization of the diet. This is the main reason for digestive disorders when an abrupt and drastic change in the diet of captive ruminants takes place. In a region such as the Kalahari desert in which the first section of this study was done, niche separation exists between the animal species. With differing diets, rumen microbial population composition is assumed to be different. This could also be influenced by chewing efficiency, rumen buffering capacity, rumen size and rate of passage. Therefore one would expect differences in the rate of digestion of a particular ration when incubated with a constant volume of rumen fluid from different animals in the same region and season.

With rumen fluid obtained from blue wildebeest and gemsbok in October, a lag in the fermentation rate is evident during the first four hours. Certain bacteria could be present in low numbers due to limited availability of a particular substrate, which might well be protein in this case, and could increase rapidly in a short time with introduction of suitable substrate. This could contribute to the overall more rapid fermentation after the first four hours. This is probably due to microbial adaptation, and was also apparent in two of the winter months when kudu rumen fluid was used.

In conclusion, *in vitro* digestibility data of plant material based on the Tilley and Terry (1963) method should not be indiscriminately extrapolated to wild ruminants. The *in vitro* digestibility of a substrate is a function of the source of rumen inoculum, time of year and the donors' feed selection.

Reference

TILLEY, J.M. & TERRY, R.A., 1963. A two-stage technique for *in vitro* digestion of forage crops. *J. Br. Grassl. Soc.* 18, 104.