S. Afr. J. Anim. Sci. 11, 287–289 (1981)

RESEARCH NOTE

THE INFLUENCE OF DIVERGENT INOCULA ON *IN VITRO* DIGESTIBILITY

Receipt of MS 05-05-1981

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(Key words: *Influence, divergent inocula,* in vitro *digestibility)*
(Sleutelwoorde: *Invloed, verskillende inokulums,* in vitro-*verteerbaarheid)*

The determination of the digestibility of forages has considerable value in the estimation of their feeding value for ruminants (Blaxter, 1960). Both *in vivo* and *in vitro* techniques can be used for the determination of digestibility. In the case of natural pastures, especially in the Karoo Region with its rich botanical composition, it is seldom possible to obtain sufficient plant material for *in vivo* methods. Where oesophageal fistulated sheep are used for sampling, only the *in vitro* method is applicable.

Concern about inoculum variability has been indicated in many reports (Engels & Van der Merwe, 1967; Kummeno, Dehority & Johnson, 1967; Calder, 1970; Minson & McLeod, 1972; Nelson, Ellzey, Montgomery & Morgan, 1972; Clark, 1975). Clark & Mott (1960) concluded that in quality comparisons among forage lines, samples of all the lines should be digested with the same batch of rumen fluid. However, this will be almost impossible in investigations conducted over time.

There is little and inconclusive evidence on the influence of incula obtained from sheep fed on shrub species, on *in vitro* digestibility determinations. Therefore, this experiment was deemed necessary.

Saltbush (*Atriplex nummularia*), Kapokbush (*Eriocephalus glaber*) and Anchor karoo (*Pentzia incana*) was harvested by hand in approximation to the parts preferred by sheep (the leafy parts with thin stems). About 3 000 kg of each plant species was collected. Lucerne hay (*Medicago sativa*) was included as a control. Harvested material was dried in the sun to a moisture content of ± 10% and then milled through a 6 mm sieve. Representative samples (± 1 kg) of each of the abovementioned fodders were collected, milled through a 1,0 mm sieve and stored in airtight containers.

Sixteen Dorper wethers fitted with rumen fistulae were divided according to body mass stratification into 4 equal groups. They were fed in indivudual feeding pens on abovementioned fodders. A preliminary period of one month was allowed before inocula were collected for *in vitro* digestibility determination.

The two-stage technique of Tilley & Terry (1963) with modifications described by Engels & Van der Merwe (1967) was used to determine *in vitro* digestibility. The fodder samples were digested individually with inocula obtained from each individual sheep. This *in vitro* digestibility trial was repeated 3 times at weekly intervals. The layout of this part of the experiment was in accordance with a randomized block design described by Snedecor & Cochran (1971).

Table 1

Chemical composition and dry matter intake (D.M.I.) of experimental feeds

Experimental feed	Chemical composition (%)				Dry matter intake of sheep $g/Wkg^{0,75}d^{-1}$		
	Crude protein	Acid detergent fibre	Ether extract	Ash			
Lucerne hay	19,62	42,6	1,38	9,7	108,6	±	$8,5^{a}$
A. nummularia	22,93	21,3	2,70	23,2	43,4	±	$10,7^{cc}$
P. incana	11,70	42,5	4,35	8,4	110,6	±	$16,3^{a}$
E. glaber	10,34	43,3	5,80	8,6	90,55	±	$12,6^{b}$

Average with the same superscript differs not significantly ($P > 0,05$), while different superscripts differs significantly ($P < 0,05$) and double superscripts differs highly significantly ($P < 0,01$) $a > b > c$

Table 2

In vivo *digestibility and effect of different inocula on* in vitro *digestibility*

Experimental feeds	Inocula				Average *in vitro* D.M.D.	Average *in vivo* D.M.D.
	Lucerne hay	*A. nummularia*	*P. incana*	*E. glaber*		
Lucerne hay	60,96 ± 2,87	61,30 ± 0,82	61,20 ± 0,98	60,96 ± 1,51	61,04 ± 1,70^{b}	52,90 ± 2,89^{b}
A. nummularia	68,78 ± 2,17	69,79 ± 1,38	68,92 ± 0,88	68,50 ± 1,21	69,04 ± 1,55^{c}	68,58± 3,16^{c}
P. incana	42,57 ± 2,91	41,74 ± 1,47	42,09 ± 1,94	40,92 ± 2,11	41,83 ± 2,19^{d}	45,91 ± 2,81^{d}
E. glaber	38,71 ± 3,56	37,78 ± 1,19	38,67 ± 1,76	38,49 ± 2,88	38,42 ± 2,47^{e}	43,02 ± 2,81^{e}
Average	52,76^{a}	52,70^{a}	52,72^{a}	52,15^{a}	52,58^{a}	55,10^{a}
	±12,92	±13,56	±12,91	±13,06	±13,40	±12,55

Significance of differences: $a \not> a$ $(P > 0,05)$

$c > b > d > e$ $(P < 0,01)$

An *in vivo* digestion trial was carried out according to a 4 x 4 latin square experimental design (Snedecor & Cochran, 1971) with 8 Merino wethers divided into 4 equal groups. A preliminary period of 3 weeks in individual feeding pens was used to accustom the sheep to the different fodders. Similarly, 3 weeks elapsed between switch over of fodders.

These digestibility trials were carried out in metabolism cages according to the method described by Niemann, Swart & Van der Merwe (1968). A seven day preliminary and 7 day collection period was used. The fodders used in the *in vivo* digestibility determination were in a pelleted form to eliminate selective consumption.

The 3 shrub species were chosen on grounds that *E. glaber* is unpalatable while *P. incana* is regarded as being palatable. *A. nummularia* was included due to its high salt content.

The chemical composition of the fodders used and average daily intakes of the Merino wethers are given in Table 1.

From Table 1 it is evident that marked differences within the chemical components existed between the fodders, all of which could have an effect on digestibility. Dry matter intakes (DMI) also reflected differences in palatability with *A. nummularia* having the lowest palatability.

The results summarised in Table 2 shows that there were no significant ($P > 0,05$) differences between mean *in vitro* organic matter digestibility, irrespective of the source of rumen inoculum with which the different fodders were fermented. This carroborates the results of Nik-Khah & Tribe (1977).

According to Table 2 the differences between average digestibilities (*in vivo* and *in vitro*) of the different forages was statistically highly significant ($P < 0,01$). *In vivo* digestibility ranged from 43,02 per cent for *E. glaber* to 68,58 per cent for *A. nummularia*. It is also evident from Table 2 that *in vitro* digestibility underestimated *in vivo* values at the lower range by up to 4,5 percentage units. Nevertheless, a high correlation ($r = 0,99$) between *in vivo* and *in vitro* digestibilities was procured, which indicates a close relationship. The resultant regression equation was $y = 10,5704 + 0,8469\ x$ ($x =$ *in vitro*- and $y =$ *in vivo* digestibility) with a standard error of prediction (Sy.x) of ± 0,16. This equation corresponds to the results of Engels & Van der Merwe (1967).

Contrary to the results of Engels & Van der Merwe (1967) a statistical significant difference ($P < 0,05$) occurred between inoculum donor sheep. As recorded in Table 2, this differences was negated when values within inoculum donor groups were pooled. Based on the standard deviations (S.D.) it was calculated (Snedecor & Cochran, 1971) that the inocula of at least 4 donor sheep should be pooled for *in vitro* digestibility determinations.

The majority of researchers concerned with inoculum variability (Bezeau, 1965; Engels & Van der Merwe, 1967; Chalder, 1970; Nelson *et al.*, 1972; Nik-Khah & Tribe, 1977) used cultivated fodders in their experiments. They found that inocula obtained from sheep on fodders low in protein, produced extensive variation in *in vitro* digestibility. The addition of nitrogen, however, reduced this variation considerably (Wilkens, 1966; Engels & Van der Merwe, 1967).

In this study divergent fodders were fed to inoculum donors. This could even enhance shifts in the rumen

micro organism population of the donor sheep to a greater extent than speculated on by Bezeau (1965) and Nelson *et al.*, (1972). However, as indicated in Table 2, S.D.'s within fodders ranged between 1,70 and 2,47 which is well within results reported by authors mentioned already. Furthermore, the S.D.'s of *in vitro* digestibility are lower than that of *in vivo* digestibility (Table 2). Therefore, it is evident that if the inoculum is fortified with nitrogen (20 mg urea per digestion tube in this study), the influence of inoculum variety on *in vitro* digestibility results is of small concern. Hence, *in vitro* digestibility values closely predict *in vivo* digestibility results.

It can be concluded that if fortified with nitrogen, divergant inocula have an insignificant effect on *in vitro* digestibility results. With sheep as inoculum donors, inocula of at least 4 sheep should be pooled for *in vitro* digestibility determinations. *In vitro* standard deviations are however, well within *in vivo* digestibility results. Due to underestimations especially at the lower digestibility ranges, it is suggested that a range of samples with known *in vivo* digestibility should be included in each *in vitro* run. By calculating a regression equation for each run, elimination of between replication variation is possible.

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