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The determination of digestibility of *Atriplex nummularia* cv. De Kock (Oldman's Saltbush) using different *in vitro* techniques

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Abstract

The main disadvantages of the rumen fluid *in vitro* technique are the cost and welfare issues of keeping cannulated animals. The purpose of the study was to find an accurate alternative *in vitro* technique to determine organic matter digestibility (OMD) of *Atriplex nummularia* supplemented with two energy sources. The *in vitro* faeces technique of El Shaer *et al*. (1987) is an easier and cheaper alternative to the classic rumen fluid *in vitro* technique of Tilley & Terry (1963), as modified by Engels & Van der Merwe (1967). The *in vitro* gas production technique of Piennaar (1994), the cellulase *in vitro* techniques of De Boever *et al*. (1986) and the modified Wageningen one, were not as accurate determining OMD as the *in vivo* technique.

Keywords: *In vitro* techniques, rumen and faeces inoculum, gas production, cellulase, *Atriplex*
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Introduction

In vivo determinations of digestibility in ruminants are expensive, labour intensive and time consuming. This creates a need for a simple, cheap and reliable laboratory technique to evaluate the nutritive value of forages for ruminant animals. The cost and animal-welfare considerations also make the use of animals less desirable. An increasing human population and demand for animal products create the need for the evaluation of new animal feedstuffs and improved varieties of traditional ones (Williams, 2000).

Most *in vitro* digestibility techniques rely on fermentation of feeds with buffered rumen fluid. In order to obtain rumen fluid, ruminally cannulated animals are required, which are expensive to maintain and in some circumstances unavailable (El-Meadaway *et al*., 1998). Another disadvantage of using rumen inocula is that a uniform diet should be fed if the inocula are to have constant activity (Akhter *et al*., 1999).

The objective of the study was to determine which of the *in vitro* techniques provide the best prediction of the organic matter digestibility (OMD) of *Atriplex nummularia* cv. De Kock, supplemented with different levels of maize and barley.

Materials and Methods

Atriplex nummularia cv. De Kock was harvested between the end of March and the beginning of April 2001, sun dried and sorted into edible and non-edible material. Edible material was defined as leaf and stems with a diameter of 6 mm and less. After sorting, the material was milled through a hammermill with a 25 mm sieve size.

Atriplex nummularia was supplemented with three levels (15%, 30%, 45%) of either maize and barley. The two energy sources used, differed in their fermentation rates. Maize is a slower fermentable and barley a faster fermentable energy source. A standard digestibility trial with five wethers was conducted to obtain the *in vivo* organic matter digestibility.

The following *in vitro* techniques were used:

a) The *in vitro* rumen fluid technique (RFT) (Tilley & Terry, 1963, as modified by Engels & Van der Merwe, 1967);
b) The *in vitro* faeces fluid technique (FFT) (El Shaer *et al*., 1987);
c) The *in vitro* gasproduction technique (GPT) (Pienaar, 1994);
d) The *in vitro* cellulase technique (CTdB) (De Boever *et al*., 1988);
e) The *in vitro* cellulase technique (CTWI) (Wageningen Institute of Animal Science, The Netherlands).

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An analysis of variance with the Proc GLM model (SAS, 1994) was used to determine the significance between the different techniques. Means and standard deviations (s.d.) were calculated. Significance of difference (5%) between means was determined with Bonferroni's test (Samuels, 1989).

Results and Discussion

Table 1 The organic matter digestibility (%) (s.d.) of *A. nummularia* cv. De Kock, supplemented with different levels of maize and barley, using different techniques

	Treatment*					
Levels	RFT	FFT	GPT	CTdB	CTWI	*In vivo*
Atriplex (*A.*) 0%	39.5^{a}(± 0.9)	38.2^{a}(± 3.3)	30.8^{b} (± 1.2)	34.0^{ab}(± 0.3)	36.9^{a}(± 1.2)	34.3^{ab}(± 10.2)
A.+15% Maize	60.8^{a}(± 0.1)	60.1^{a}(± 2.5)	37.2^{b}(± 0.1)	44.2^{b}(± 1.6)	48.8^{b}(± 0.1)	54.7^{a}(± 7.0)
A.+ 15% Barley	62.4^{a}(± 10.6)	62.3^{a}(± 1.5)	35.4^{b}(± 0.1)	46.7^{b}(± 1.5)	47.1^{b}(± 0.5)	61.3^{a}(± 5.8)
A.+ 30% Maize	64.9^{a}(± 2.0)	63.2^{a}(± 0.9)	50.1^{b}(± 2.4)	56.6^{b}(± 1.0)	48.7^{b}(± 0.3)	55.7^{b}(± 14.1)
A.+ 30% Barley	69.6^{a}(± 0.4)	68.1^{a}(± 6.4)	44.6^{b}(± 0.7)	53.1^{b}(± 0.4)	47.1^{b}(± 0.4)	62.4^{a}(± 8.4)
A.+ 45% Maize	68.3^{a}(± 0.1)	66.3^{a}(± 1.8)	58.0^{b}(± 0.7)	61.3^{ab}(± 0.9)	59.0^{b}(± 1.2)	64.0^{ab}(± 8.5)
A.+ 45% Barley	72.5^{a}(± 10.1)	73.3^{a}(± 4.7)	54.1^{b}(± 0.5)	61.2^{ab}(± 0.7)	58.7^{ab}(± 0.9)	67.0^{a}(± 7.0)

*See text for detail techniques
[abc]Row means with common superscripts do not differ (P > 0.05)

No differences (P > 0.05) were found between the rumen- and faeces inoculum *in vitro* techniques, but they did differ from the gas production and cellulase techniques (P < 0.05). There was also no difference between the gas production and cellulase techniques (P > 0.05). The OMD of the RFT and FFT techniques did not differ from the *in vivo* OMD values (P < 0.05).

There are several possibilities for the difference between the *in vivo* and *in vitro* OMD. 1. Practical mistakes could have been made. 2. The simulation of the rumen motility *in vitro* is often difficult and it may be that all the feed particles did not have the same exposure to the microorganisms, as it would have in the rumen of an animal. The different rumen pools are also not fully represented *in vitro*. 3. The fermentation characteristics and microbial constitution of the rumen inocula differ between the animal used for the *in vivo* digestibility trial and the animals used for rumen inocula collection. 4. With *in vivo* digestibility the time of digestion is not known, and therefore the time of rumen and gastric digestion *in vitro* could have been too long or too short.

Conclusion

The results of this study demonstrated that both the rumen- and faeces inoculum *in vitro* techniques can be used to determine the OMD of *A. nummularia* supplemented with an energy concentrate (slower and faster fermentable) up to 45%. This confirms that the *in vitro* faeces technique of El Shaer *et al*. (1987) is an easier and cheaper alternative to the classic rumen fluid *in vitro* technique of Tilley & Terry (1963), as modified by Engels & Van der Merwe (1967) to determine the OMD of ruminant feeds. The gas production and cellulase *in vitro* techniques resulted in lower OMD values than the *in vivo* technique.

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