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The use of in vitro techniques in routine ruminant feed evaluation

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Abstract. This study determined the nutritive value of some browse assessed bythe in vitro dry matter digestibility and kinetics of gas production at several incubation times (till 144 h) with or without the addition of polyethylene glycol (PEG 6000). Species varied widely in their *in vitro* dry matter digestibility (IVD, 33-61%), asymtotic gas production (A, 187-291 ml/g DM) and fractional rate of gas production (c, 0.030-0.047/h). Addition of PEG to the incubation medium increased the asymptotic gas production (A) by 51% for *P. lentiscus* and the parameter c by 60% for *M. communis*. Effect of PEG on the volume of gas produced was generally greater at 6 and 12 h of incubation. Irrespective of the incubation time, the greatest increase was observed with *A. unedo, E. arborea, M. communis* and *P. lentiscus*. Based on their *in vitro* digestibility and gas production characteristics, *P. angustifolia*, *M. communis* and *C. villosa* were superior in quality to *P. lentiscus*, *Q. suber*, *A. unedo* and *E. arborea*. The results suggest that the *in vitro* gas production may be appropriate to assess browse with relatively high phenolic concentrations, such as *P. lentiscus*, *E. arborea* and *A. unedo*.

Key words: Shrubs – Gas production – Antinutritional factors – Tannins.

Utilisation des techniques* in vitro *dans l'évaluation de routine des aliments des ruminants

Résumé. *Cette étude détermine la valeur nutritionnelle de certains arbustes fourragers en déterminant la digestibilité de la matière sèche et la cinétique de production de gaz* in vitro *(jusqu'à 144 h) et ce en présence et en absence de polyéthylène glycol 6000 (PEG 6000). Les espèces étudiées ont montré une large variabilité pour la digestibilité* in vitro *de la matière sèche (DIMS, 33-61%), le potentiel de production de gaz (A, 187-291 ml/g MS) et le rythme fractionnel de la production de gaz (c, 0,03-0,047/h). L'addition du PEG au milieu d'incubation s'est traduite par une augmentation du potentiel de production de gaz (A) de 51% (*P. lentiscus*) et du paramètre c de 60% (*M. communis*). Dans le cas de* C. villosa *et* Q. suber *l'addition du PEG s'est accompagnée par une réduction du paramètre c. L'effet du PEG sur le volume de gaz produit a été généralement élevé après 6 et 12h d'incubation. Indépendamment du temps d'incubation l'effet du PEG a montré la plus forte augmentation chez* A. unedo, E. arborea, M. communis *et* P. lentiscus. *Sur la base des données sur la DIMS et des caractéristiques de production de gaz,* P. angustifolia, M. communis *et* C. villosa *sont de bonne qualité nutritionnelle par rapport à* P. lentiscus, Q. suber, A. unedo *et* E. arborea. *Ces résultats suggèrent que la technique de production de gaz* in vitro *pourrait être appropriée pour les arbustes fourragers relativement riches en composés phénoliques tels que* P. lentiscus, E. arborea *et* A. unedo.

Mots-clés. *Arbustes – Production de gaz* – In vitro – *Tannins.*

I – Introduction

Despite their abundance in the Tunisian rangelands and their evergreen foliage throughout the year, many browse species, such as *Erica* spp., *Quercus* spp. and others, have been undervalued. The prejudice against these species is attributed mainly to the insufficient knowledge about their potential feeding value. *In vitro* incubation techniques are valuable tools to estimate digestibility and kinetics of ruminal fermentation of roughages. Moreover, quantification of tannins seems to be an important factor in the process of evaluating true nutritive value of shrubs and their suitability as feed sources in the feeding systems (Kumar and

D'Mello, 1995). Recently, it has been suggested that occurrence of tannins and their effect on the kinetics of *in vitro* fermentation can be assessed using the gas production technique coupled with the use of a tannin-binding agent such as polyethylene glycol 6000 (PEG 6000). The objectives of the present study were to estimate the nutritive value of some Tunisian shrubs using *in vitro* techniques and determine the biological activity of tannins with the gas production technique in presence of PEG.

II – Material and methods

Leaves and stems of seven browse species namely *Arbutus unedo* L., *Calicotome villosa* (Poir) Link, *Erica arborea* L., *Pistacia lentiscus* L., *Myrtus communis* L., *Phillyrea angustifolia* L. and *Quercus suber* L., were collected in spring from the uplands in the province of Taaref in the delegation of Nefza (northwest of Tunisia). The selection of the species was based in the available information about their palatability and preference by small ruminants and on their relative abundance in the studied area. In the laboratory, leaves, twigs and small stems (Ø< 2 mm) were separated by hand, oven-dried at 40°C and finely ground (1 mm screen). Chemical composition and phenolic compounds were studied previously (Ammar *et al.,* 2005).

For the *in vitro* studies rumen fluid was obtained from four rumen cannulated Merino sheep fed 1 kg alfalfa hay daily and with free access to water and mineral/vitamin licks. A sample of rumen content was collected before the morning meal in thermos flasks and taken immediately to the laboratory where it was strained through four layers of cheesecloth and kept at 39°C under a CO_2 atmosphere.

1. In vitro digestibility

A culture medium containing macro- and micro-mineral solutions, a bicarbonate buffer solution and resazurin was prepared as described by Goering and Van Soest (1970). Rumen fluid was then diluted into the medium in the proportion 1:4 (v/v). Samples (250 mg) were weighed out into polyester bags (two bags per sample), 5-L glass recipients with a plastic lid provided with a single-way valve which avoids the accumulation of fermentation gases. Then the buffered rumen fluid was anaerobically transferred into the incubation jars (2-L per jar). The jars were placed in incubator (DAISY, ANKOM) at 39°C, with continuous rotation. After 48 h of incubation, bags were rinsed under cold tap water and in a washing machine (short washing cycle with cold water), dried at 60°C for 48 h and weighed to determine *in vitro* dry matter Digestibility (IVD)

2. In vitro gas production

For the assessment of the kinetics of gas production and the biological activity of tannins (using PEG 6000), the technique proposed by Theodorou *et al.* (1994) was followed. A sample (300 mg) of each browse species was weighed in triplicate into serum. Buffer solutions and rumen liquor/buffer (1:4) were prepared as described above, and 50 ml of rumen/buffer mixture were anaerobically dispensed into each bottle, with the addition of either 2 ml of distilled water or 2 ml of an aqueous solution of PEG (25 g/ 100 ml, for an intended addition of 500 mg PEG per bottle). All the bottles were crimped and placed in the incubator at 39°C and the volume of fermentation gas released was measured at 3, 6, 9, 12, 16, 21, 26, 31, 36, 48, 60, 72, 96, 120 and 144 h post-inoculation using a pressure transducer (Theodorou *et al.,* 1994). In order to estimate the kinetics of gas production, data of the cumulative gas volume produced were fitted to the exponential model proposed by France *et al.* (2000) **¡Error! No se pueden crear objetos modificando códigos de campo.**, where G (ml/g) denotes the cumulative gas production at time t; A (ml/g) is the asymptotic gas production; c (h^{-1}) is the fractional fermentation rate and L (h) is the lag time.

One-way ANOVA was performed on with browse species as the only source of variation. The Bonferroni test was used for the multiple comparison of means.

III – Results and discussion

The foliage of *Ph. angustifolia* had the highest values of IVD (0.613) and rate of gas production (0.047 h^{-1}) and *C. villosa* had the highest value of G24 (211 ml/g DM) and asymptotic of gas production, A (343 ml/g DM) (Table 2). However, *M. communis* had the slowest fermentation rate (0.030 h^{-1}). The volume of gas produced at 6 h of incubation (G06) was higher (>70 ml/g DM) only for *C. villosa* and *Ph. angustifolia* (Table 1). This was expected since gas volume measured after a few hours of fermentation mainly reflect the fermentation of highly soluble feed fractions. However, further increase of the gas production in relation to the incubation time, maybe is due to the decrease of the release of tannins (Makkar *et al*., 1995) and consequently increase the volume of gas produced. When compared with *P. lentiscus*, a tannin-rich shrub, all parameters of gas production were generally higher in *C. villosa* and *M. communis* (Table 1). Nonetheless, fractional fermentation rate suggests that tannins and indigestible components of the cell wall affect the rate of gas production to a littler extent than the others parameters.

Table 1. In vitro digestibility (IVD) and parameters of gas production kinetics of browse foliage

Species	IVD g/g DM	G06h ml/g DM	G24h ml/g DM	A ml/g DM	c /h
A. unedo	0.468bc	53b	156c	263c	0.037b
C. villosa	0.609a	73a	211a	343a	0.040b
E. arborea	0.381de	42d	126d	217d	0.036b
M. communis	0.525b	49c	151c	292b	0.030c
Ph. angustifolia	0.613a	71a	197b	291b	0.047a
P. lentiscus	0.330e	46cd	127d	190e	0.046a
Q. suber	0.422cd	44d	122d	187e	0.044a
SEM	0.0126	0.8	1.9	3.0	0.0007
P-value	<0.001	<0.001	<0.001	<0.001	<0.001

SEM Standard error of the mean.
a,b,c,d,e Means with different letter within the same column are significantly different (P<0.05).

It is well established that condensed tannins at high levels may depress ruminal fermentation of feed, reducing microbial activity and digestibility. In the current study the higher levels of tannins present in *A. unedo* were accompanied generally by higher kinetics of fermentation than *Q. suber*, which revealed lower tannin content (Ammar *et al*., 2005). This finding may indicate that *Q. suber* possess a mechanism for reducing microbial degradation in the rumen which is independent of tannins. Up to date, few studies have studied the effect of tannins on plant palatability. *E. arborea*, for example, an evergreen species, is preferred by sheep and goats through the year in spite of its high tannin content. The percentage increase in gas production represents the effect of tannins, the higher the biological activity of tannins on rumen microbes, the higher the increase in gas production in presence of PEG. The largest increment was observed to *P. lentiscus* (86, 80, 71, 60 and 51% for G06, G12, G24, G48 and asymptotic of gas production respectively) (Table 2). *M. communis* presented the highest increase in the fermentation rate (60%). Irrespective of the browse species, the magnitude of the increase of the volume of gas produced peaked at 6 h and decreased progressively with the incubation time. The effect of PEG on the volume of gas produced decreased progressively with the incubation time. Such findings indicate that microbes can adapt or counteract some of the antinutritive effects.

Addition of PEG to tannin-free plants (i.e., *C. villosa* and *Ph. angustifolia*) did not increase the *in vitro* gas production and may result in negative effects by decreasing the efficiency of microbial synthesis. Our results strongly indicate that addition of PEG is advantageous if the tannin content of the feed is high to the extent that it depresses microbial activity and digestibility of

feeds drastically. Although total condensed tannin content was higher in *E. arborea* than in *M. communis* and *A. unedo* (Ammar *et al.,* 2005), effect of PEG on kinetics of gas production from these two latter species was larger than that from *E. arborea*.

Table 2. Increase (%) of the in vitro gas production parameters with the PEG presence

Species	ΔG06	ΔG12	ΔG24	ΔG48	ΔA	Δc
A. unedo	66b	58b	44b	29b	14b	55a
C. villosa	-4c#	-3d#	-2d#	0d#	3c#	-7e
E. arborea	57b	51b	40b	27b	14b	44b
M. communis	64b	56b	44b	27b	8bc	60a
Ph. angustifolia	6c#	6cd#	5cd#	4cd#	3c#	4d#
P. lentiscus	86a	80a	71a	60a	51a	27c
Q. suber	12c	13c	13c	14c	15b	-2de
SEM	3.1	2.9	2.5	2.1	1.8	1.4
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

SEM Standard error of the mean.
a,b,c,d,e Means with different letter within the same column are significantly different (P<0.05).
Effect of PEG not significant (P>0.05).

IV – Conclusion

Based on the data of kinetics of *in vitro* fermentation, the browse species had the following ranking: *C. villosa*>*P. angustifolia*>*M. communis*>*A. unedo*>*Q. suber*>*E. arborea*>*P. lentiscus.* However, although methods used here for screening the nutritional potential of browse plant species have some advantages, the practical feeding experiment with the target ruminant species is the most suitable method. Further studies are therefore recommended to determine whether the observed superiority in the nutritional value of *C. villosa*, *P. angustifolia* and *M. communis* could be translated into improved animal performance. The apparently low nutritive value of *A. unedo*, *E. arborea* and *P. lentiscus* makes them less suitable as an alternative feeding stuff in ruminant diets.

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