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in

Ben Salem H. (ed.), López-Francos A. (ed.).
Feeding and management strategies to improve livestock productivity, welfare and product quality under climate change

Zaragoza : CIHEAM / INRAT / OEP / IRESA / FAO
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 107

2013
pages 121-125

Chemical composition and *in vitro* fermentation characteristics of range species growing in Central Tunisia

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Abstract. Chemical composition and *in vitro* fermentation characteristics were determined on 15range(herbaceous and shrubby) species collected in a postural area from the region of Kairouan (Central Tunisia) during the grazing period (end of winter and spring). The *in vitro* fermentation parameters were determined using 100 ml glass syringes containing the plant material and inoculum and incubated for 96 hours. Correlations between chemical parameters, calculated *in vitro* organic matter digestibility ($IVOMD_{24h}$) and metabolisable energy (ME) were determined. Crude protein (CP) content was highest in *Rosedaalba* (184 g/kg DM) and *Medicago minima* (175 g/kg DM) and the lowest in *Rosemarinus officinalis* (58 g/kg DM) and *Globularia alypum* (61 g/kg DM). The NDF contents varied widely between the studied species and ranged from 273 g/kg DM in *Rosedaalba* to 607 g/kg DM in *Marrubium vulgare*. The same wide variation was observed for ADF since it varied from 169 to 502 g/kg DM respectively in *Rhus tripartita* and *Artemisia herba alba*. Lignin content (ADL) was highest in *Pistacia lentiscus* (239 g/kg DM) and lowest in *Calendula arvensis* (49 g/kg DM). Asymptotic gas production (A) varied from 82.7 ml in *Chrysanthemum coronarium* to 26.7 ml in *Artemisia herba alba*. Positive correlations were found between CP content and $IVOMD_{24h}$ (r= 0.74, P<0.0001) and also ME (r= 0.76, P<0.0001). While, negative correlations were found between $IVOMD_{24h}$ and NDF (r= -0.58, P<0.0001), ADF (r= -0.65, P<0.0001) and ADL (r= -0.72, P<0.0001) contents. The same trend was noted with ME (r= -0.58, -0.63, -0.73, P<0.0001, respectively for NDF, ADF and ADL). It was concluded that the range species available in the studied area presented a wide nutritional variability, thus they could have a complementary role for small ruminants grazing. Considering the laborious *in vivo* approach in pasture conditions, the in vitro technique may considerably contribute into evaluating such diversity of resources. Secondary compounds should be considered in order to improve accuracy of predictive equations.

Keywords. Nutritive value – *In vitro* fermentation – Pasture – Small ruminants.

Composition chimique et paramètres de fermentation* in vitro *des espèces pastorales de la Tunisie Centrale

Resumé. *La composition chimique et les caractéristiques de la fermentation* in vitro *ont été déterminées pour 18 espèces pastorales (herbacées et arbustives) collectées dans une zone pastorale de la région de Kairouan (Tunisie centrale) au cours de la période de pâturage (fin d'hiver et printemps). La composition chimique a été déterminée et les paramètres de fermentation* in vitro *ont été mesurés dans des seringues en verre de 100 ml pendant 96 heures. Nous avons établi des corrélations considérant, d'une part,les valeurs calculées de la digestibilité de la matière organique* in vitro *($DIVMO_{24h}$) et de l'énergie métabolisable (EM) et d'autre part la composition chimique des espèces étudiées. Les teneurs en matières azotées totales (MAT) les plus élevées ont été enregistrées pour* Roseda alba *(184 g/kg MS) et* Medicago minima *(175 g/kg MS) et les plus basses pour* Rosmarinus officinalis *(58 g/kg MS) et* Globularia alypum *(61 g/kg MS). La teneur en NDF variait considérablement entre 273 g/kg MS pour* Roseda alba *et 607 g/kg MS pour* Marrubium vulgare*. La même variation a été observée pour la teneur en lignocellulose (ADF), qui est passée de 169 à 502 g/kg MS, respectivement pour* Rhustripartita *et* Artemisia herba alba*. La teneur en lignine (ADL) était la plus élevée pour* Pistacia lentiscus *(239 g/kg MS) et la plus basse pour* Calendula arvensis *(49 g/kg MS). L'asymptote (a) de la production de gaz varie de 82,7 ml pour* Chrysanthemum coronarium *à 26,7 ml pour* Artemisia herba alba*. Des corrélations positives significatives ont été trouvées entre la teneur en MAT et la $DIVMO_{24h}$ (r = 0,74 ; P <0,0001) et également avec l'EM (r = 0,76 ; P <0,0001). Cependant, des corrélations négatives ont été trouvées entre la $DIVMO_{24h}$ et la NDF (r = -0,58; p <0,0001), l'ADF (r = -0,65, p <0,0001) et l'ADL (r = -0,72, p <0,0001). La même tendance a été notée avec l'EM (r = -0,58 ; -0,63 et -0,73 ; p <0,0001, respectivement pour NDF, ADF et ADL). Il a été conclu que les espèces*

disponibles dans la zone étudiée présentent une grande variabilité nutritionnelle qui pourrait représenter une certaine complémentarité pour le pâturage des petits ruminants. Compte tenu des difficultés et du coût des approches in vivo *dans les conditions de pâturage, la technique* in vitro *peut considérablement contribuer à l'évaluation de la diversité des ressources. Les composés secondaires devraient être considérés pour améliorer la précision des éventuelles équations prédictives.*

Mots-clés. *Fermentation* in vitro *– Valeur alimentaire – Espèces pastorales – Petits ruminants.*

I – Introduction

In Tunisia, grazing pasture represents the major feeding system for small ruminants, mainly in the central regions. A large biomass diversity composed of native species is available in these regions. Rangeland improvement and rehabilitation and grazing management inquire the nutritive characterization of grass and shrubs species (Ben Salem *et al*., 1994; Nefzaoui *et al*., 1995).The study aimed to determine the nutritional characteristics of range species from the region of Kairouan (central Tunisia),and to contribute into the establishment of feeding value database in this specific feeding system.

II – Materials and methods

1. Plant material

Fifteen range specieswere collected from the region of Kairouan (semi-arid, central region of Tunisia) in March 2008. Samples from each species were taken from different sites and pooled to make a global sample. The dry matter (DM) was determined at 105°C in a forced-air oven and a part of each sample was dried at 40°C during 48h and then ground to pass through 1 mm screen and stored for chemical analysis and *in vitro* determinations.

2. Animals and measurements

Two adult "Noire de Thibar breed" sheep (average age and live weight respectively 24 months and 48.5 kg) with rumen cannula were used for *in vitro* determinations. They were housed in individual pens and received twice per day 70 g DM $kg^{-0.75}$of a diet composed of 70% oat-vetch hay and 30% of barely grains on dry matter (DM) basis. Samples (300 mg DM) of each species were incubated in 100 ml glass syringes according to the technique of Menke and Steingass (1988).The incubation medium (30 ml) was a mixture of rumen fluid and Menke buffer solution (1:1). Gas production was measured at 2, 4, 6, 12, 24, 36, 48, 72 and 96 h of incubation. Diets were incubated in triplicate and two successive incubations were carried out.

3. Chemical analysis

Feeds were analyzed for dry matter (DM), ash and crude protein (CP) contents (AOAC, 1984). Cellwall composition (NDF, ADF and ADL) in feeds were determined as described by Van Soest *et al*. (1991).

4. Calculation and statistical analysis

Gas production kinetic was fitted using the non-linear model of France (2000):

$G=b*(1-e^{-k(t-L)})$

where: "G" is the gas production at time t; "b" asymptotic gas production, "k" the fractional rate

of gas production and "L" lag-phase. Parameters were calculated using NLIN procedure of SAS (SAS, 1996). *In vitro* organic matter digestibility at 24h ($IVOMD_{24h}$) and metabolizable energy (ME) were calculated according to the specific equations of Menke and Steingass (1988).

III – Results and discussion

Chemical composition of the range species is presented in Table 1. *Reseda alba* showed the highest CP content (184 g/kg DM), whereas the lowest concentrations were observed in *Rosmarinus officinalis* (58 g/kg DM). *Artemisia herba alba* and *Marrubium vulgare* L. were highest in NDF (637 and 607 g/kg DM, respectively). The same trend was observed for ADF contents (502 and 421 g/kg DM, respectively in *Artemisia herba alba* and *Marrubium vulgare* L.). The range species evaluated in the current work exhibited a wide nutritional variability, suggesting that they could have a complementary role for goat feeding and grazing. Our results were close to those found by several authors (e.g. Ben Salem *et al*., 1994, 2000; Gasmi-Boubaker *et al*. (2009).

Table1. Chemical composition of feeds (g/kg DM)

	Ash	CP	NDF	ADF	ADL
Artemisia campestris L.	58	98	561	452	126
Artemisia herba alba	67	99	637	502	216
Calendula arvensis	156	125	306	222	49
Chrysanthemum coronarium	81	140	335	257	62
Globularia alypum	53	61	425	328	125
Marrubium vulgare L.	86	121	607	421	135
Medicago minima	149	175	334	234	86
Olea europea	59	89	390	238	142
Picris echioides	220	161	435	267	141
Pistacia lentiscus	50	93	378	315	239
Reseda alba	114	184	273	178	56
Rhamnus lycioides	177	164	289	179	59
Rhus tripartita	134	140	321	169	199
Rosmarinus officinalis	62	58	389	301	167
Thymus capitatus	102	88	409	326	226

In vitro fermentation parameters of feeds are presented in Table 2. Asymptotic gas production (b) ranged from 26.7 and 82.7 ml respectively in *Artemisia herba alba* and *Chrysanthemum coronarium.* The Lag time (L) was shortest and the rate of gas production was highest in *Reseda alba*. The Metabolizable energy ranged between 1122 kcal/kg DM in *Rosmarinus officinalis* and 3099 kcal/kg DM in *Rhamnus lycioides.*

As an example, Figure 1 shows the *in vitro* gas fermentation of *Calendula arvensis.* Gas productions increased with increased incubation time and tended to be stabilized from 48 h of incubation.

The main correlations between *in vitro* parameters and chemical composition are presented in Table 3. Negative correlations were found between EM and NDF (r=-0.58, P<0.01), ADF (r=-0.65, P<0.01), ADL (r=-0.72, P<0.01) and L (r=-0.24, P<0.01). The same trend is observed between $IVOMD_{24h}$and NDF (r=-0.58, P<0.01), ADF (r=-0.63, P<0.01), ADL (r=-0.72, P<0.01) and L (r=-0.29, P<0.01). Positive correlation was detected between $IVOMD_{24h}$ and k (r=0.55, P<0.01).These results are in line with those reported by Ammar *et al.* (2005).

Table 2. Parameters of *in vitro* fermentation

	b (ml)	k ($10^{-3}h^{-1}$)	L(h)	$IVOMD_{24h}$ (%)	ME (kcal/kg DM)
Artemisia campestris L.	37.1	85	0.3	42.6	1801
Artemisia herba alba	26.7	55	2.58	36.3	1179
Calendula arvensis	62.5	85	0.46	63.6	2886
Chrysanthemum coronarium	82.7	95	0.18	70.5	2908
Globularia alypum	48.6	57	0.4	43	1801
Marrubium vulgare L.	36.4	72	0.73	44.6	1427
Medicago minima	61.8	91	0.48	65.5	2935
Olea europea	55.9	59	0.06	48.6	2027
Picris echioides	41.3	72	0.97	58.5	2895
Pistacia lentiscus	34.1	40	1.80	36.2	1227
Reseda alba	73.6	136	0	72.1	3071
Rhamnus lycioides	58.4	130	0.30	67.6	3099
Rhus tripartita	49.6	55	0.98	53.4	1606
Rosmarinus officinalis	27.7	90	1.01	34.4	1122
Thymus capitatus	54.3	76	1.83	53.4	1704

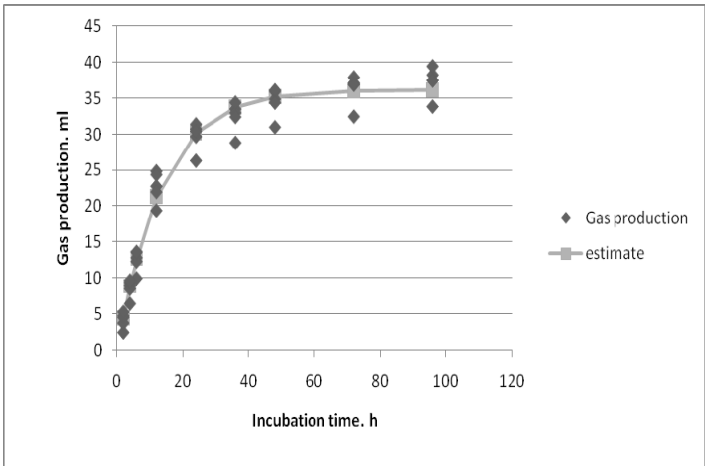

Fig. 1. Gas production from *in vitro* fermentation of *Artemisia campestris* L.

Table 3. Main correlation coefficients between chemical composition and *in vitro* parameters.

	CP	NDF	ADF	ADL	b	k	L
ME	0.76**	-0.58**	-0.65**	-0.72**	0.64**	0.55**	-0.24**
$IV_{24h}OMD$	0.74**	-0.58**	-0.63**	-0.72**	0.81**	0.55**	-0.29**

**P<0.01

IV – Conclusions

It was concluded that the range species evaluated in the current work presented a wide nutritional diversity, thus they could exhibit a complementary role for small ruminants grazing.

Considering the laborious *in vivo* approach in pasture conditions, the *in vitro* procedure may considerably contribute into evaluating such diversity of resources. However, the secondary compounds should be considered in order to improve accuracy of predictive equations.

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