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# EFFECT OF POLYETHYLENE GLYCOL ON *IN VITRO* GAS PRODUCTION AND DIGESTIBILITY OF TANNIN CONTAINING FEEDSTUFFS FROM NORTH AFRICAN ARID ZONE

## [EFECTO DEL POLIETILENGLICOL EN LA PRODUCCIÓN DE GAS IN VITRO Y DIGESTIBILIDAD DE ALIMENTOS TANINIFEROS DE LA ZONA ÁRIDA DEL NORTE DE AFRICA]

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**SUMMARY**

The influence of tannins present in arid zone forages from North Africa: *Aristida plumosa*, *Danthonia forskahlii*, *Astragalus gombiformis*, *Genista saharae*, two date palm fractions (leaves and racemes), and vetch-oat hay taken as control on *in vitro* gas production and *in vitro* organic matter digestibility (IVOMD) was evaluated. Chemical analysis revealed the low nutritional quality of these forages. They were high in NDF, ADF and lignin (679.5, 455.7 and 86 g/kg DM, respectively) and low in nitrogen (< 12 g/kg DM) except for *Astragalus* that had 20 g N/kg DM. Phenolic compounds (total phenols, total tannins and total condensed tannins) were 61.8, 49.1 and 36.2 g/kg DM for palm leaves followed by *Astragalus*, racemes, *Genista*, *Aristida* and *Danthonia*. Gas production ranged between 55.2 and 152.6 mL/g DM whereas IVOMD ranged between 21 and 56.5%. Addition of PEG resulted in an overall increase in both gas production (20.2%) and IVOMD (30.7%), with the exception of *Danthonia* and *Aristida*. The largest increment for gas production was recorded for *Aristida* (low tannins content). However, the higher increase in IVOMD was noted for racemes, *Astragalus* and palm leaves (high tannins content). The variable responses among forages studied suggest that factors other than phenolic compounds also affect *in vitro* fermentation.

**Key words:** *in vitro* gas production; North African forages; polyethylene glycol; *in vitro* digestibility.

**RESUMEN**

Se evaluó la influencia de los taninos presentes en forrajes de zonas áridas del norte de África: *Aristida plumosa*, *Danthonia forskahlii*, *Astragalus gombiformis*, *Genista saharae*, dos fracciones de la palma (hoja y panicula), y heno de avena como control para la producción de gas *in vitro* y la digestibilidad *in vitro* (IVOMD). Los forrajes son de baja calidad nutricional ya que contienen elevadas concentraciones de FDN, FDA y lignina (679.5, 455.7 y 86 g/kg MS respectivamente), y un bajo contenido de N (<12 g/kg MS) excepto *Astragalus* cuyo contenido de N fue de 20 g /kg MS. Los compuestos fenolicos (fenoles totales, taninos totales y taninos condensados) fueron 61.8, 49.1 y 36.2 g/kg MS para las hojas de palma, seguidos por la panicula de *Astragalus*, *Genista, Aristida* y *Danthonia.* La producción de gas fluctuó de 55.2 a 152.6 mL/g MS mientras que la IVOMD fue de 21 a 56.5%. La adición de PEG resultó en un incremento de la producción de gas (20.2%) e IVOMD (30.7%) con la excepción de *Danthonia* y *Aristida*. El mayor incremento de la producción de gas se registró en *Aristida* (bajo contenido de taninos). Sin embargo, el mayor incremento en IVOMD fue observado en las paniculas de *Astragalus* (alto contenido de taninos). La respuesta variable entre los forrajes sugiere que existen otros factores en adición a los compuestos fenolicos que afectan la fermentación *in vitro*.

**Palabras clave:** Producción de gas *in vitro*; forrajes del norte de África; PEG; digestibilidad *in vitro*.

## INTRODUCTION

Animal production in Algeria, particularly in arid regions, is almost exclusively based on pasture of native plants. These plants can be classified into two main groups (Longuo et *al*., 1989): ephemeral plants, which germinate and remain green for only a few weeks after rain, and perennial plants, characterized by a slow vegetative cycle with a growing period from March to June (Haddi et *al*., 2003). Moreover, the arid regions are represented in part by oasis where the cultivation of date palm trees is preponderant. Local farmers use date palm fractions, principally discarded dates, leaves and racemes for ruminant feeding supplementation (Genin et *al*., 2004). Most of these forages contain antinutritive factors such as tannins in response to their harsh environmental conditions. However, no studies have been done on the effect of tannins present in this type of fodders on in vitro fermentation.

The in vitro gas production method is a relatively simple and inexpensive tool to study potential effects, mechanisms of action and fate of phytochemicals in the rumen (Makkar, 2005). This method, coupled with polyethylene (PEG) used as a specific binding agent, provides useful information on the biological activity of tannins (Ammar et *al*., 2004a). However, the *in vitro* gas production must be completed by other measures of end-products fermentation such as the amount of degraded matter for obtaining a more complete information. This study was conducted for assessing the effect of tannins, using PEG (MW 4000), on in vitro gas production and degradability of four plants collected from the South-East of Algeria and two date palm fractions, comparatively to vetch-oat hay taken as control.

## MATERIAL AND METHODS

### Study area

Forages were collected from the administrative districts of El-Oued, located in the South-East of Algeria. El-Oued region is situated at 6°53’E and 32°20’N. Its average altitude above sea level is 67 m. The climate of the region is arid with annual mean rainfall of 75 mm, and mean temperature comprised between 1°C in January and 43°C in July.

### Forage samples collection and processing

Experimental feedstuffs consisted of four autochthonous North African species and two date palm fractions, widely utilized by local farmers. The plants were selected based on herdsmen knowledge, that are consumed by dromedary, goats and sheep.

The gramineous (*Aristida plumosa*: locally named *Ksiba* and *Danthonia forskahlii:* locally named *Sfar*) and leguminosea (*Genista saharae*: locally named *Merck* and *Astragalus gombiformis*: locally named *Foulet El Ibel*) plants were harvested at maturity (June 2004) and flowering (March 2004) stages, respectively. The edible plant samples (leaves, stems and flowers) were hand plucked and chopped to 2-cm length. The date palm fractions were: racemes (stems floral without dates) and leaves (leaflets and rachis). The racemes were sampled from the dates conditioning factory. For leaves, the samples consisted on leaves removed at senescence stage from date palm trees. Vetch-oat hay, taken as control, was provided by ITELV (Technical Institute of Breeding, Ain Mlila, Algeria). The samples were dried at 60°C in forced air oven for 48-h, except samples for tannins determination that were sun dried. The forages were then ground to pass a 1-mm sieve and used for chemical analysis, in vitro gas production and in vitro digestibility.

### Chemical analysis

Samples were analysed for dry matter (DM) and organic matter by the methods of the AOAC (1990; method ID 942.05). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (ADL) were determined using the reagents described by Van Soest et al. (1991). The crude protein content of plants and crude protein bound to NDF fraction (NDICP) were determined by the Kjeldhal procedure.

### Phytochemical analysis of antinutritive factors

The phytochemical analysis, based on colorimetric reactions, was carried out according to the procedures described by Larrahondo (1985) and Rosales et al. (1989). The dried samples (15 g) were extracted with methanol solution (90%, 30 mL) and ether (30 mL) for 40 min. The extraction was repeated three times, and the mixture was filtered. At the end of the extraction procedure and decantation, two phases were obtained; the lower layer is the methanol-water (polar phase) and the top non-polar fraction being formed with ether.

For determination of saponins, 9 mL of water were added to 1 mL of the methanol fraction and then filtered. 1 mL of this solution was vigorously shaken in a small test tube for 30 seconds. After 15 minutes, the height of foam was measured, giving an indication of the levels of saponins in the forage using the following criteria: < 5 mm absence of saponins, 5-9 mm low content of saponins, 10-14 mm medium content of saponins and > 15 mm high content of saponins.

The phenolic compounds were determined by addition of two or three drops of ferric chlorure ($FeCl_3$) to 1 mL of methanol extract which was diluted to 50% by distilled water. The change in colour indicated the presence of phenolic compounds as follows: none change absence of phenolic compounds, dark blue presence of phenols or hydrolysable tannins, and dark green presence of condensed tannins

For steroids, 10 mL of aqueous extract treated with chlorhydrique acid to drop pH to 2.0 units, were added to 10 mL of ether extract. After evaporation, 0.5 mL of chloroform and 0.5 mL anhydrous acetic acid were added. The addition of 1 to 2 mL of sulfuric acid induced the apparition of greenish blue colour, which indicates the presence of steroids.

**Quantitative analysis of phenolic compounds**

**Total phenols.** Analysis of phenolic compounds was carried out in three replicates. Dried plant material (200 mg) was extracted with 10 mL of acetone (70% v/v) and solution was subjected to ultrasonic treatment for 20 min at room temperature. The content was centrifuged (4°C, 10 min, 3000 g) and the supernatant was kept on ice until analysis. The pellet was treated a second time as described above.

Total phenols (TP) were estimated by the Folin-Ciocalteau reaction (Makkar et al., 1993). A calibration curve was prepared using tannic acid. Total phenols were calculated as tannic acid equivalent and expressed as tannic acid eq-g/kg DM.

**Tannins.** For Total condensed tannins (TCT), the extract (0.5 mL) was treated with n butanol-HCl (3 mL, 95%) in the presence of ferric ammonium sulfate (0.1 mL). Reagents were heated in a boiling water bath for 60 min. Absorbance was read at 550 nm. TCT were expressed as leucocyanidins equivalents following the equation:

$$TCT = \frac{A_{550\ nm}\ x\ 78.26\ x\ dilution\ \ factor}{weight\ \ of\ sample\ \ on\ DM}$$

Where: $A_{550\ \mathrm{nm}}$ is absorbance at 550 nm assuming that the effective $E^{1\%,\ 1\ \mathrm{cm},\ 550\ \mathrm{nm}}$ of leucocyanidin is 460 (Porter et al., 1986).

Total tannins (TT) were determined as the difference in total phenolics (measured by Folin-Ciocalteau reagent) before and after treatment with insoluble polyvinylpyrrolidone (Makkar et al., 1993). They were also determined according to the radial diffusion assay (Hagerman, 1987). Petri dishes were prepared with agarose and acetate buffer (pH= 5, 0.05 M). The acetone extract (40 µL) was added to wells (6 mm diameter) bored in the solidified agarose and the Petri dishes were placed in an incubator at 30°C, for 72 h. The diameters of the resulting rings were measured. The amount of tannins is proportional to the square of the diameter of the ring. A calibration curve was prepared using tannic acid. Values were expressed as tannic acid eq-g/kg DM.

***In vitro* study**

**Animals and management.** Rumen fluid, used as inoculum for in vitro fermentation, was obtained from three sheep fitted with permanent cannulae, that were fed a daily ration of 700 g vetch-oat hay (chemical composition illustrated in table 1) and 300 g of concentrate (barley 58%, wheat bran 38%, mineral and vitaminic premix 1%, NaCl 1% and limestone 2%) divided into two equal meals at 8:00 and 16:00 h. The sheep had free access to water throughout the experiment.

**Polyethylene glycol (peg) tannin bioassay**

Effect of polyethylene glycol (PEG) on the in vitro fermentation of feedstuffs was determined using glass syringes (100 ml) as described by Menke et al. (1979). The gas production parameters and metabolisable energy (ME) were determined in incubation with 200 mg of sample, but in vitro organic matter digestibility (IVOMD) and partitioning factor (PF: organic matter truly degraded (mg) and gas volume produced (mL) ratio) were measured in incubation with 350 mg of forage (Blümmel and Becker, 1997). The two incubations were made at the same time, in absence or presence of 1 g of PEG (MW, 4000) (Makkar et al., 1995). Rumen fluid was obtained from the three sheep in the morning 1 hour before feeding, flushed with $CO_2$, filtered through four layers of gauze and mixed with an anaerobic mineral solution as described by Makkar et al. (1995). The buffer solution was prepared without addition of nitrogen ($NaHCO_3$, 39 g/L). A portion (40 mL) of the buffered rumen fluid was transferred into the syringes and incubated at 39°C in an isothermal incubator equipped with a rotor, which run continuously at 9 rpm. All incubations were in triplicate. Gas production readings were recorded after 3, 6, 9, 24, 48, 72 and 96 h for the first incubation. Whereas for the second incubation, the fermentation was stopped at 24 h. Syringe contents were quantitatively transferred into a beaker by rinsing syringes with a total of 50 mL of neutral detergent solution (double strength; Blümmel and Becker, 1997a) and refluxed 1 h. The residue was filtered through a sintered glass crucible No.2 (pore size 40-90 µm). Residual organic matter was determined as outlined earlier.

**Calculations and statistical analysis**

For the first incubation, values of gas production, recorded at 96-h and corrected for blank incubation that contained only rumen fluid plus buffer solution, were treated by the exponential model of Orskov and Mc Donald (1979): $y = a + b\ (1 - \exp^{-ct})$, where y (mL/g DM) is the gas production at time t, a (mL/g DM) is the gas production from the immediately degradable fraction, b (mL/g DM) is the gas production from the insoluble fraction, a+b (mL/g DM) is the potential gas production, c ($h^{-1}$) is the rate of gas production, and t (h) is the incubation time. The metabolisable energy (ME) was calculated using the equation as follows:

ME (MJ/kg DM) = 2.20 + 0.136 GP + 0.057 CP
(Makkar and Becker, 1996)

Where: CP is crude protein expressed as g/kg DM and GP is mean gas production.

Data on chemical composition and tannins were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS (1990), and were analysed based on the statistical model $Y = \mu + F_i + e$, where y is the general observation on nutrient compounds and tannins, μ is the general mean common for each parameter under investigation, $F_i$ is the effect of substrate on the observed parameter, and e is the standard error term. Data on in vitro gas production, metabolisable energy, in vitro true organic matter digestibility, and partitioning factor were analysed based on the statistical model $Y = \mu + F_i + P_j + (F\times P)_{ij} + e$, where, *Y* is the general observation, $F_i$ is the effect of substrate on the observed parameters, $P_j$ is the effect of PEG, $(S\times P)_{ij}$ represents the interaction effects of substrate and PEG, and e is the standard error term common for all observations. Differences between treatments were compared using student's Newmann-Keuls test, and were considered statistically significant at $P < 0.05$. Standard errors of means were calculated from the residual means square in the analysis of variance.

## RESULTS

### Chemical analysis

Chemical composition of the substrates is shown in Table 1. There was a wide variation in all nutrient compounds of the forages. Except *G. saharae*, racemes and vetch-oat hay, all forages had high ash content (> 100 g/ kg DM). The highest value was noted for *A. gombiformis* (231.7 g/kg DM) and the lowest for *G. saharae* (36.4 g/kg DM). The crude protein content was also highly variable, ranging from 25 g/kg DM in racemes to 125 g/kg DM in *A. gombiformis*, compared to vetch-oat hay (67.5 g/kg DM). Substantial quantities of CP were associated with NDF in *D. forskahlii* and date palm fractions, decreasing available crude protein significantly. The highest contents of NDF and ADF were found in *D. forskahlii* and the lowest ones were recorded for date palm leaves and vetch-oat hay (586.1 and 327.7 g/kg DM, respectively). All the samples had high lignin content, except vetch-oat hay. The highest value corresponding to *G. saharae* (142.4 g/kg DM) and the lowest value to vetch-oat hay (43.6 g/kg DM).

Phytochemical analysis shows that feedstuffs contained antinutritive factors such as steroids, saponins, and phenols (Table 2). The quantitative analysis of phenolic compounds is also illustrated in Table 2. The lowest values were recorded for the *Poaceae* family, whereas the highest levels were observed in the *Fabaceae* family and date palm fractions. The TP ranged from 4.5 g/kg DM in vetch-oat hay to 61.8 g/kg DM in date palm leaves, which had also the higher TT and TCT fraction, 49.1 and 36.2 g/kg DM, respectively. *A. plumosa* and *D. forskahlii* did not contain TCT. The highest PPC value was also recorded for date palm leaves followed by racemes, whereas, the other forages had not any PPC action. The ranking of forages according to tannins content was as follows: palm leaves > *A. gombiformis* > *G. saharae* = racemes > *A. plumosa* > *D. forskahlii* > vetch-oat hay. There was a negative correlation between NDF and TEP (r = 0.661), and TET (r = 0.632), but there was no significant correlation between phenolic compounds and ADF, lignin and crude protein. The protein precipitation capacity (PPC) and TT were positively correlated with TCT (r = 0.759, and 0.856, respectively, $P < 0.05$).

### Effect of polyethylene glycol on *in vitro* gas production (GP) and metabolisable energy (ME)

The gas production over 24-h and ME, without and with PEG, are presented in Table 3. In absence of PEG, the lowest gas volume produced was recorded for racemes (55.28 mL/g DM) and the highest value was noted for *A. gombiformis* (152.6 mL/g DM). The ranking of feedstuffs on the basis of gas production was as follows: *A. gombiformis* > vetch-oat hay > *G .saharae* > *D. forskahlii* > *A. plumosa* > palm leaves > racemes. As expected, addition of PEG increased gas volume after 24-h in all forages except for *D. forskahlii*. The highest increment was recorded for *A. plumosa* (54.3 units) and the lowest for *A. gombiformis* (9.10 units). In presence of PEG, the ranking of feedstuffs on the basis of gas production was as follows: vetch-oat hay > *A. gombiformis* > *G. saharae* > *A. plumosa* > *D. forskahlii* > palm leaves = racemes. The result indicate that *A. plumosa* tannins, whose concentration were low, reduced gas production significantly, but that of *A. gombiformis* appeared to be less sensitive to PEG. In presence of PEG, the gas production from *A. plumosa*, *A. gombiformis* and *G. saharae* come closer to vetch-oat hay. Furthermore, in presence of PEG the differences in gas production between leaves and racemes became insignificant. Besides, there were several unexpected results. In palm leaves, PEG addition had small effect compared to *A. plumosa*, despite their high tannins content which was detected by all chemical and biological assays. Similarly, PEG increased gas from *A. plumosa*, but not from *D. forskahlii,* although the two forages had approximately similar tannins content. Addition of PEG resulted also in an increase of ME content. Estimated ME was influenced (P<0.05) by substrate and PEG addition (P<0.05). However, the interaction of substrate and PEG effects was not significant (P>0.05). Racemes had the lowest ME content (5.14 MJ/kg DM) in the absence of PEG compared to *A. gombiformis* that had the highest ME of 13.42 MJ/kg DM. The presence of PEG caused a little increase in ME in all forages. The highest increment was recorded for *A. plumosa* (1.48 units), comparatively to *A. gombiformis* (0.3 units) for which the PEG addition induce a low increase in ME that was already higher.

**Effect of polyethylene glycol on gas production parameters**

There were effects of substrate, PEG addition, and interaction of substrate and PEG (P<0.05) on potential gas production (a+b), but addition of PEG did not influenced the rate of gas production (Table 4). Vetch-oat hay had the higher potential gas production, both in the presence and the absence of PEG. Whereas, racemes had the higher rate of gas production, both with and without PEG. For some forages (leaves, *D. forskahlii* and *A. gombiformis*), rate of gas production was slightly decreased in the presence of PEG.

**Effect of polyethylene glycol on *in vitro* truly OM digestibility (IVOMD) and partitioning factor (PF)**

There was an effect of substrate, PEG addition, and interaction of substrate and PEG addition on IVOMD (P<0.05) (Table 5). The IVOMD values ranged from 21.0% in racemes to 56.4% in vetch-oat hay in incubation without PEG. The ranking of forages on the basis of IVOMD was: vetch-oat hay > *A. plumosa* = *G. saharae* > *D. forskahlii*= palm leaves > *A. gombiformis* > racemes. Addition of PEG increased highly IVOMD in feedstuffs with high tannin contents (racemes, *A. gombiformis* and palm leaves, 25.7, 24.9 and 20.0 units, respectively) but moderately in forages with low tannin contents (vetch-oat hay, *D. forskahlii* and *A. plumosa*, 3.57, 3.25, 1.20 units, respectively). In presence of PEG, the ranking of forages became as follows: vetch-oat hay = palm leaves > *A. gombiformis* = *G. saharae* > racemes = *A. plumosa* = *D. forskahlii*. Partitioning factor (PF) was influenced (P<0.05) by substrate, and interaction of substrate and PEG (Table 5). In the absence of PEG, the PF values ranged from 2.56 mg/mL in *A. gombiformis* to 7.20 mg/mL in palm leaves. Addition of PEG decreased PF in two forages (*A. plumosa* and palm leaves) indicating that PEG addition in these forages promote gas production but not organic matter digestibility. Whereas, addition of PEG did not alter PF in *G. saharae, D. forskahlii* and vetch-oat hay, but induced an increase in PF values for *A. gombiformis* and racemes.

Table 1. Chemical composition (g/kg DM) of selected feedstuffs from arid zone of North Africa.

| Substrates | DM | OM | CP | NDF | ADF | Lignin (sa) | NDICP |
|---|---|---|---|---|---|---|---|
| *A. plumosa* | 846.6$^{c}$ | 885.4$^{d}$ | 74.4$^{b}$ | 747.9$^{b}$ | 413.8$^{e}$ | 64.5$^{d}$ | 23.3$^{c}$ (30.91) |
| *D. forskahlii* | 899.8$^{b}$ | 888.3$^{d}$ | 60.6$^{d}$ | 824.4$^{a}$ | 562.3$^{a}$ | 79.2$^{c}$ | 39.5$^{a}$ (62,85) |
| *A. gombiformis* | 551.7$^{e}$ | 768.1$^{e}$ | 125.0$^{a}$ | 614.9$^{d}$ | 445.2$^{d}$ | 78.1$^{c}$ | 40.2$^{a}$ (32.34) |
| *G. saharae* | 894.0$^{b}$ | 963.6$^{a}$ | 74.4$^{b}$ | 621.9$^{c}$ | 499.1$^{c}$ | 142.4$^{a}$ | 24.5$^{c}$ (33.02) |
| Palm leaves | 896.3$^{b}$ | 890.5$^{d}$ | 59.4$^{e}$ | 586.1$^{e}$ | 422.1$^{e}$ | 97.1$^{b}$ | 33.1$^{b}$ (55.32) |
| Racemes | 923.4$^{a}$ | 936.1$^{c}$ | 25.0$^{f}$ | 745.1$^{b}$ | 519.9$^{b}$ | 97.3$^{b}$ | 13.3$^{d}$ (53.60) |
| Vetch-oat hay | 891.1$^{b}$ | 942.2$^{b}$ | 67.5$^{c}$ | 616.2$^{e}$ | 327.7$^{f}$ | 43.6$^{e}$ | 31.2$^{b}$ (45.80) |
| S.E.M. | 0.83 | 0.25 | 0.46 | 2.3 | 8.4 | 4.36 | 1.87 |

DM, dry matter ; OM, organic matter ; CP, crude protein ; NDF, neutral detergent fibre assayed without a heat stable amylase and expressed inclusive of residual ash ; ADF, acid detergent fibre expressed inclusive residual ash ; NDICP, crude protein bound to NDF fraction and its concentration relative to CP (g/100 CP) represented between parenthesis; ADL, lignin determined by solubilisation of cellulose with sulphuric acid ; S.E.M., standard errors of means ; $^{a, b, c, d, e, f}$ means with different superscripts within a same column are significantly different (P < 0.05).

Table 2. Phytochemical analysis of antimicrobial factors and quantitative analysis of phenolic compounds (g/kg DM, standard equivalent) of selected feedstuffs.

| | Phytochemical analysis | | | Quantitative analysis | | | |
|---|---|---|---|---|---|---|---|
| Substrates | Steroids | Saponins | Phenols | TP | TT | TCT | PPC |
| *A. plumosa* | - | - | + | 6.8[d] | 4.4[d] | 0.00[e] | 0[c] |
| *D. forskahlii* | - | + | + | 5.4[e] | 3.2[e] | 0.00[e] | 0[c] |
| *A. gombiformis* | + | + | + | 34.0[b] | 21.3[b] | 4.0[c] | 0[c] |
| *G. saharae* | - | + | + | 24.5[c] | 18.2[c] | 0.70[e] | 0[c] |
| Palm leaves | - | + | + | 61.8[a] | 49.1[a] | 36.2[a] | 55.45[a] |
| Racemes | - | - | + | 24.5[c] | 18.2[c] | 21.3[b] | 32.25[b] |
| Vetch-oat hay | - | - | + | 4.5[f] | 2.2[f] | 1.80[d] | 0[c] |
| S.E.M. | | | | 0.5 | 0.5 | 0.5 | |

TP, total phenols; TT, total tannins ; TCT, total condensed tannins ; PPC, protein precipiting capacity ; S.E.M., standard errors of means ; [a, b, c, d, e, f] means in the same column affected with different letters are significantly different ($P < 0.05$) ; -, absence of colour or foam in the test tube which reveals that the feedstuff don't contain an antinutritional factor ; +, colour development or foam apparition in the test tube that indicate the presence of antinutritional factor

Table 3. Corrected gas volume (mL/g DM) after 24 h and metabolisable energy (MJ/kg DM) in the absence and presence of polyethylene glycol of the selected feedstuffs.

| | Effect of PEG on GP response | | Effect of PEG on ME response | |
|---|---|---|---|---|
| Substrates | - PEG | + PEG | - PEG | +PEG |
| *A. plumosa* | 84.2[ef] | 138.5[c] | 8.73[c] | 10.21 |
| *D. forskahlii* | 101.1[e] | 91.5[e] | 8.40[d] | 8.14 |
| *A. gombiformis* | 152.6[abc] | 161.7[ab] | 13.42[a] | 13.72 |
| *G. saharae* | 118.4[d] | 142.2[c] | 9.51[bc] | 10.32 |
| Palm leaves | 71.1[f] | 87.7[ef] | 7.52[e] | 7.97 |
| Racemes | 55.28[g] | 85.86[ef] | 5.14[f] | 5.49 |
| Vetch-oat hay | 146.6[bc] | 168.4[a] | 10.04[b] | 10.65 |
| Species | * | | * | |
| PEG treatment | * | | * | |
| Species x PEG | * | | NS | |
| S.E.M. | 1.50 | | 0.40 | |

ME, metabolisable energy ; PEG, polyethylene glycol ('+': with, '-': without); [a, b , c, d, e, f, g] means with different letters in the same row are significantly different ($P < 0.05$); *, denotes significant effect of species, PEG treatment and their interaction on in vitro gas production and ME of feedstuffs ($P < 0.05$) ; NS, non significant ($P > 0.05$).

Table 4. Effect of polyethylene glycol on in vitro gas production parameters over 96 h of the selected feedstuffs.

| | a (mL/g DM) | | a + b (mL/g DM) | | c ($\%h^{-1}$) | |
|---|---|---|---|---|---|---|
| Substrates | - PEG | + PEG | - PEG | + PEG | - PEG | + PEG |
| *A. plumosa* | 6.81 | 11.7 | 122.4[g] | 190.2[bc] | 5.09[ef] | 5.14[ef] |
| *D. forskahlii* | -19.2 | -19.46 | 151.2[ef] | 160.4[de] | 4.87[ef] | 4.51[f] |
| *A. gombiformis* | 9.73 | 3.53 | 176.9[cd] | 191.01[bc] | 8.22[bc] | 7.66[bcd] |
| *G. saharae* | 29.1 | 20.95 | 112.4[f] | 170.75[d] | 6.64[bcde] | 7.70[bcd] |
| Leaves | -5.9 | 32.21 | 80.7[i] | 113.4[g] | 7.67[bcd] | 6.16[cde] |
| Racemes | 12.4 | 7.56 | 65.2[j] | 94.73[h] | 8.41[b] | 12.64[a] |
| Vetch-oat hay | 6.81 | 19.0 | 194.65[b] | 213.7[a] | 5.72[def] | 6.43[bcde] |
| Significance | | | | | | |
| Substrate | | | * | | * | |
| PEG treatment | | | * | | NS | |
| Substrate x PEG | | | * | | * | |

a, the gas production corresponding to the soluble fraction ; a + b, potential gas production ; c, rate of gas production ; [a, b, c, d, e, f, g, h, I, j] means in the same row without a common letter differ ($P < 0.05$) ; *, denote significant effect of species, PEG treatment and their interaction at $P < 0.05$ ; NS, non significant ($P > 0.05$).

## DISCUSSION

Chemical composition of plants from arid zone of North Africa that are resistant to drought and salinity is poorly documented. High ash content is a characteristic of desertic plants (Bokhari et al.,1990; Stringi et al.,1991), ash content was also relatively high (>100 g/kg DM) in most forages tested in this work except for *G. saharae*, racemes and vetch-oat hay. This was probably due in all of them to the contamination of aerial part of plants with sand. In the present study, CP content was particularly low (< 75g/kg DM) in almost all forages in agreement with reported data (Bahman et al., 1997; Pascual et al., 2000; Genin et al., 2004; Ramirez et al., 2004 and Ammar et al., 2004b). This may be due to the influence of soil type, environmental conditions and genotypic characteristics, factors that affect the nutritional proprieties of forages. Whereas, the relatively high CP in *A. gombiformis* (> 100g/kg DM) indicate its possible use as protein supplement. Crude protein associated with NDF fraction appeared to be higher in palm fractions and *D. forskahlii* which reduces the available nitrogen for ruminal microbiota, and implies that these forages would require a protein supplement for their use in ruminant feeding. All forages contained high NDF, ADF and lignin. These results were similar to that observed by Pascual et al. (2000) and Genin et al. (2004) for date palm fractions, by Ramirez et al. (2004) for *Aristida* genus, and by Ammar et al. (2004b) for *Genista* genus. The high cell wall content recorded could be due to the arid zone climate. In general, high temperature and low rainfall tend to increase cell wall polysaccharides and to decrease the soluble carbohydrates (Pascual et al., 2000).

Concentration of phenolic compounds varied widely among plant species. The lowest values were recorded for the *Poaceae* family, whereas the highest levels were observed in the *Fabaceae* family and date palm fractions, consistently with the results pointed out in the literature (Tisserand, 1990). The PPC values in all forages except date palm fractions were very weak. These results were not related to the quantity of tannins in the samples. This could be due to the fact that radial diffusion method, based on the measure of the potential biological activity of tannins in feeds, depend upon binding strength of tannins and their mode of binding to protein (Frazier et al., 2003), whereas chemical methods, based on chemical properties of tannins, indicate only the chemical nature of tannins (Silanikove et al., 1996).

Table 5. Effect of polyethylene glycol on in vitro organic matter digestibility and partitioning factor of forages sampled from the North African arid zone.

| Substrates | IVTOMD | | PF | |
|---|---|---|---|---|
| | - PEG | + PEG | - PEG | + PEG |
| *A. plumosa* | 45.58$^{c}$ | 46.78$^{c}$ | 5.93$^{b}$ | 3.92$^{c}$ |
| *D. forskahlii* | 41.13$^{d}$ | 44.38$^{c}$ | 5.82$^{b}$ | 5.12$^{b}$ |
| *A. gombiformis* | 26.83$^{e}$ | 51.73$^{b}$ | 2.56$^{d}$ | 3.47$^{c}$ |
| *G. saharae* | 44.96$^{c}$ | 50.94$^{b}$ | 5.31$^{b}$ | 5.29$^{b}$ |
| Palm leaves | 39.65$^{d}$ | 59.72$^{a}$ | 7.20$^{a}$ | 6.15$^{b}$ |
| Racemes | 21.08$^{f}$ | 46.80$^{c}$ | 3.93$^{c}$ | 5.40$^{b}$ |
| Vetch-oat hay | 56.44$^{a}$ | 60.01$^{a}$ | 5.48$^{b}$ | 5.28$^{b}$ |
| Significance | | | | |
| Species | * | | * | |
| PEG treatment | * | | NS | |
| Species x PEG | * | | * | |
| S.E.M. | 1.92 | | 0.53 | |

IVOMD (g/100g DM), in vitro true organic matter digestibility ; PF (mg/mL), partitioning factor ; a, b, c, d, e, f means in the same row with different letters are significantly different ($P < 0.05$) ; * denotes significant effect of species, PEG treatment and their interaction at $P < 0.05$ ; NS, non significant ($P > 0.05$).

The inclusion of PEG in fermentation media of the feedstuffs resulted in a marked increase in in vitro gas production with all forages except *D. forskahlii* for which this parameter was reduced in presence of PEG. The increases in gas production when samples were incubated with PEG were also reported for different forages by other authors (Baba et al., 2002; Rubanza et al., 2005 and Singh et al., 2005). As in this study, Singh et al. (2005) have also noted that addition of PEG reduced the gas production for two forages: *Leucaenea leucocephala* (-18.59 and –18.56%) and paddy straw (-7.39 and –6.52%) at 24 and 48h, respectively. The highest responses on in vitro gas production due to the inclusion of PEG in *A. plumosa* could be due to the inhibition of tannin effects. All tannin assays showed that *A. plumosa* and *D. forskahlii* had low content in phenolic compounds. However, *A. plumosa* tannins appeared to be more active than that of *D. forskahlii* because they produced large responses to PEG in the gas test. The discrepancies between the two forages may be likely attributed either to the limited ability of PEG to completely inhibit the negative effects of tannins (Baba et al., 2002; Frutos et al., 2004), which depends mainly both on stereochemistry and chemical structure of tannins, or to other factors that may be more important than tannins in limiting fermentation (Ndlovu and Nherera, 1997), which in the case of *D. forskahlii* could be the limited available nitrogen for ruminal microbiota, the higher NDF, ADF and lignin contents, and the saponins detected in this species.

Min et al. (2003) reported that condensed tannin levels of 20-40 g/kg DM produce beneficial effects. Getachew et al. (2002) concluded in an other study that samples containing total phenols and total tannins (tannic acid equivalent/kg DM) up to 40 and 20 g/kg DM, respectively, are not expected to induce an increase in gas production on addition of PEG. In contrast, almost all forages in this study had TT and TCT within the two ranges but gives a positive responses to PEG supplementation. These results showed that effects of tannins seem to be depended on several factors such as forage species, chemical nature and structure of tannins, biochemical interaction among tannins and proteins, than the tannins level itself. On the other hand in palm leaves, PEG addition had small effect despite their relatively high tannin content.

Gimárãez-Beelen et al. (2006) have reported that PEG treatment of three Brazilian legumes reduced the astringency by approximately 70%. Thus, the increase in gas production, in racemes in presence of PEG could possibly due to an increase in the available nutrient to ruminal microbiota especially nitrogen. For the leguminous plants and especially *G. saharae,* the effect of PEG on in vitro fermentation could be probably masked by the high lignin content in this forage.

The effect of PEG addition is more pronounced on potential gas production, measured at 96 h of incubation. The effects of tannins on nutrient degradability depends essentially on the formation of complexes between tannins and the components of diets, primarily proteins and to a lesser extent with aminoacids, polysaccharides and minerals, as well as on their effects on the microbial population and on its enzymatic activity (Mc Sweeney et al., 2001). For racemes, addition of PEG resulted in an important increase of both potential gas production and rate of gas production. This result could suggest that tannins in this case are binding to fibres and the presence of PEG increased microbial plant adhesion and/or the fibrolytic microbial activity. However, the PEG supplementation induces a decrease in rate gas production in some forages, especially date palm leaves. This result has also been reported by Frutos et al. (2004) and Gimárãez-Beelen et al. et al. (2006). The latter authors have noted that for species, which the rate of gas production is reduced, the bacteria colonisation is restricted. This could suggest that complexes forming between tannins and PEG generate steric obstruction which do not permit and/or limit the fixation of adherent bacteria to the feeds.

In absence of PEG, *A. gombiformis* had the highest gas production, and the lowest IVOMD and PF values. Whereas, *A. plumosa* had lower gas production, and higher IVOMD and PF values. For *A. gombiformis*, the lowest IVOMD could be explain by the fact that tannins binding to proteins form complexes which are largely insoluble in ND solution, thus forming precipitates which will overestimate the undegradable fraction (Makkar et al., 1995). This situation could also suggest that fermentation process and gas production are probably affected by tannins in a complex fashion. In contrary to results obtained by Makkar et al. (1998), PEG addition in this study resulted mainly in an overall increase in IVOMD of 30.7% compared with an overall increase in gas production of 20.2%. This result indicate that effect of PEG addition improved IVOMD at the cost of gas production, which lead certainly in an improvement in microbial protein synthesis. As has been reported by Baba et al. (2002), the PEG addition resulted in decrease of PF values. However for some forages (racemes and *A. gombiformis*), addition of PEG caused an increase in PF values. The same observations have been also reported by Singh et al. (2005) for two forages (*F. roxburghii* and *R. pseudoacacia*). This was due to increased substrate degradability with lower gas production. Blümmel et al. (2003) suggested selection of forages for high degradability but proportionally low gas production.

The theoretical range for PF values for tannins free plants was suggested by Blümmel et al. (1997b) to be between 2.75 and 4.41. PF values of five forages used in this study were higher to the theoretical maximum value. According to Blümmel et al. (1997b), plants with high PF are in general highly digestible and the values correlate well with dry matter intake in ruminants. Thus, these results could suggest that these forages had a potential nutritive value which tends to enhance microbial synthesis rather than gas production.

## CONCLUSIONS

On the basis of in vitro fermentation without PEG addition results, the two leguminosea forages *A. gombiformis* and *G. saharae* were judged to be nutritionally good, followed by *A. plumosa* and *D. forskahlii*. However, the weak fermentability of date palm fractions limits their utilisation by ruminants. Addition of PEG inactivated effects of tannins at different levels in forages studied. In vitro fermentation of *D. forskahlii* was not influenced by PEG inclusion despite their tannin level comparatively similar to *A. plumosa,* in which the effect of PEG is more pronounced. These discrepancies between the two forages could be due to the limited available nitrogen for ruminal microbiota, the higher NDF, ADF and lignin content detected in *D. forskahlii*. At same, the effect of PEG in *G. saharae* was probably concealed by its high lignin content. However for date palm fractions, the inclusion of PEG is clearly detectable. These results demonstrated that the in vitro fermentation of these substrates was not only associated to the quality and proportion of tannin, but other plant factors also affect in vitro gas production and mask the effect of phenolic compounds in the bioassay test.

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