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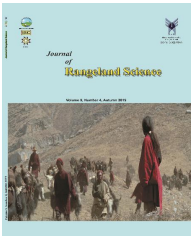

Research and Full Length Article:

Comparing Chemical Composition and Digestibility of Pedicels and Palm Leaves as a Source for Livestock Feeding by *in vitro* and *in situ* Techniques

Souhil Boufennara[AC]*, Lyas Bouazza[A], Samir Medjekal[B], Khaled Rahal[A], Ivan Mateos[C], Maria Jose Ranilla[C], SecundinoLopez[C]

[A] Department of Cellular and Molecular Biology, Faculty of Nature Sciences, University Abbes Laghrour of Khenchela, 40000. Khenchela, Algeria *(Corresponding author), Email: bouffenara@yahoo.fr

[B] Department of Microbiology and Biochemistry, Faculty of Sciences, Med Boudiaf University of M'sila, Bp166, 28000, M'sila, Algeria

[C] Instituto de Ganadería de Montaña, CSIC-Universidad de León, Departamento de Producción Animal, Universidad de León, 24007 León, Spain

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Abstract. The aim of this study was to determine the chemical composition and *in vitro* digestibility, and to estimate the *in vitro* fermentation parameters of palm leaves and pedicels from Ghars palm variety. A rumen *in situ* technique was also used to examine *In vitro* Digestible Dry Matter (IDDM), *In vitro* Digestible Crude Protein (IDCP), and *In vitro* Digestible Neutral Fiber (IDNF), and rate and extent of gas production. Vetch-oat hay was taken as a reference feed material. The samples were collected in Tolga district (southeast Algeria). TheCrude Protein (CP) content of the plant species was low for pedicels and palm leaves (33 and 60 g kg^{-1} DM, respectively). The highest content of total extractable phenols, tannins and condensed tannins was observed in palm leaves whereas pedicels showed lower concentrations. *In vitro* digestibility and *in situ* DM disappearance were slightly different for the examined forages. Analogue trends were observed for the *in vitro* fermentation kinetics estimated from the gas production curves. Pedicels showed the highest DM effective degradability (ED; assuming a passage rate of 0.03 h^{-1}) whereas palm leaves seemed to be a poorly degradable material with an ED coefficient of 0.39 and 0.14 g g^{-1}DM, respectively. Despite the moderate CP and high fiber content along with *in vitro* digestibility and *in situ* DM disappearance found in pedicels, in comparison with vetch oat hay degradation, it indicated that this plant could have a greater nutritional value. Dry matter disappearance after 144 h of incubation was negatively correlated with phenolic compounds and total extractable tannins, suggesting that the *in vitro* techniques can be appropriate for detecting the presence of anti-nutritional substances in shrubs.

Key Words: Chemical composition, *In vitro* digestibility, *In situ* technique, Tannins, Palm date

Introduction

The date palm (Phoenix dactylifera L.) is a hardy monocot tree adapted to the arid parts of the world and constitutes the oldest fruit crops grown in the arid regions of the Arabian Peninsula, North Africa, and the Middle East. This tree which constitutes the basis of the oasis agriculture makes a wide range of agricultural by-products available traditionally used for domestic purposes, and is the main source of food for the local Saharan population and feed for their livestock (Boufennara *et al*., 2016), particularly in the areas with long dry period and harsh environmental conditions as the Mediterranean regions, despite the fact that their feed quality is not as high as that of herbaceous species (Papanastasis *et al*., 2004).

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): short-live 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 and ruminants reared in those regions may be handicapped to cover maintenance requirements with only natural vegetation without any additional feed supply with the result of reduced performance. Under these conditions, the use of date palm by-products in animal diets could counteract in part the shortage of animal feed resources and subsequently increase milk and meat production (Boufennara *et al*., 2016).

The anti-nutritive effect of dry leaves of the date palm has been studied in animal models of ruminants and it seems that both reduced digestibility and toxicity may limit the potential of this plant as a feed supplement (Deffairi and Arhab, 2016). Moreover, some of these plants hold anti-nutritional secondary compounds (phenolics, tannins) with potential adverse effects such as inhibition of rumen microbial fermentation as well as reduced feed degradability and animal performance (Waghorn and McNabb, 2003). These compounds can impair the digestive utilization of the feed ingested by the animal. Biological assays to estimate the rate and extent of ruminal digestion of these fibrous feedstuffs, especially those containing secondary compounds provide comprehensive information on their potential nutritive value for ruminants.

The nutritional value of date palm residues has been extensively studied due to their high availability in the oasis countries where date production is valuable and essential. Both the energy and protein values of these by-products are low as compared to that of cereal straw (Arbouche *et al*., 2008). Although these resources gain increasing significance as the nutritional value of grass drops, they never reach a prominent position in the diet because of their low CP and high fiber concentration and low digestibility (Cabiddu *et al*., 2000).

The nutritive benefit of palm by-products can be determined by their chemical composition (Aregheore, 2000) or by a combination of chemical constituents and gas released on incubation of feeds in an *in vitro* medium containing rumen microbes (Menke and Steingass, 1988). Digestibility may be directly determined *in vivo* or estimated using *in vitro* procedures, which are cheaper and more convenient. Indeed, the *in vitro* digestibility procedures and the gas fermentation technique are engaging tools that provide such information and have also been proposed to determine the biological effect of tannins contained in feedstuffs (Ammar *et al*., 2005).

The objective of this work was to determine the chemical composition, *in vitro* digestibility, *in situ* dry matter disappearance and to estimate the *in vitro* fermentation parameters of palm leaves and pedicels.

Materials and Methods

Forage and roughage material

Palm leaves and pedicels were collected from Ghars palm variety in Tolga district (southeast Algeria). Samples were clipped with scissors and taken immediately to the laboratory, oven-dried at 50 ºC (Makkar, 2003) and subsequently ground to pass through a 1 mm screen.

Chemical analysis

Dry Matter (DM, method ID 934.01), ash (method ID 942.05) and Crude Protein (CP, method ID 954.01) contents were determined by the methods of AOAC (2000). Neutral and Acid Detergent Fibre (NDF and ADF, respectively) and sulphuric Acid Detergent Lignin (ADL) were determined with the ANKOM fibre analyser as described by Van Soest *et al.* (1991). Sodium sulphite, but not α-amylase, was added to the solution for the NDF determination. Both fibre fractions were expressed including residual ash.

Phenolic compounds were extracted by the procedures described by Makkar (2003). Total Extractable Phenols (TEP) were determined according to the method of Julkunen-Tiitto (1985) using the Folin-Ciolateau reagent and tannic acid as standard. Total Extractable Tannins (TET) was calculated indirectly after adsorption of TEP to insoluble polyvinylpyrrolidone reagent, and measuring the remaining total phenols (or non-precipitable phenols) in the supernatant. Free Condensed Tannins (FCT) was measured in the extract using the butanol–HCl assay (Porter *et al.*, 1986) with the modifications of Makkar (2003) and using purified quebracho tannin as standard. The Bound Condensed Tannins (BCT) was measured in the solid residue remaining after extraction of phenolic compounds. Concentration of phenols and tannins were expressed in g tannic acid equivalent kg^{-1}DM whereas the concentration of condensed tannins was expressed in g quebracho equivalent kg^{-1} DM. All chemical analyses were performed in triplicate.

Animals and rumen fluid extraction for in vitro and in situ studies

Four mature Merino sheep (body weight 49.4 ± 4.23 kg) fitted with a permanent ruminal cannula (60 mm diameter) were used for the extraction of rumen fluid or *in situ* incubation of nylon bags. Animals were fed with lucerne hay*ad libitum* (167 g CP, 502 g NDF, 355 g ADF and 71 g ADL kg^{-1} DM) and had free access to water and mineral/vitamin block. Samples of rumen contents were withdrawn prior to morning feeding, transferred into thermos flasks and taken immediately to the laboratory where rumen fluid was strained through various layers of cheesecloth and kept at 39 ºC under a constant flow of CO_2.

In vitro digestibility

In vitro dry matter digestibility was determined using the ANKOM-DAISY procedure (Boufennara, 2012) following two different approaches proposed by Tilley and Terry (1963), and the one described by Van Soest *et al*. (1966). Both techniques were carried out separately in different incubations.

A culture medium containing macro- and micro-mineral solutions, a bicarbonate buffer solution and resazurin was prepared as described by Menke and Steingass (1988). Samples of plant material (400 mg) were weighed into artificial fibre bags (size 5 cm × 5 cm, pore size 20 µm) which were heat-sealed and placed in incubation jars. After 48 h of incubation in buffered rumen fluid, samples were dried to estimate *in vitro* dry matter loss (ivDMloss) after 48 h incubation. Then, bags used to measure *in vitro* digestibility by the original method of Tilley and Terry (1963) were subjected to a 48 h acid pepsin–HCl digestion, and the dry residue remaining in the bag was considered as the apparently indigestible DM to estimate

InVitro Digestibility of Tilley and Terry (IVD-TT). According to Van Soest (1994), the extraction with the neutral detergent removes bacterial cell walls and other endogenous products and can be considered as a determination of the True *InVitro* Digestibility (TIVD). With each procedure, each browse sample was incubated in triplicate with one bag per sample incubated in each jar and rumen fluid from each of the four sheep being incubated separately in each of the four jars.

In vitro gas production kinetics

The technique described by Theodorou *et al*. (1994) was used to obtain gas production profiles. Ground samples (500 mg) were incubated in 50 ml of diluted rumen fluid (10 ml mixed rumen fluid + 40 ml medium prepared under a CO_2constant flow) in 120 ml serum bottles. Volume of gas produced was recorded at several incubation times (3, 6, 9, 12, 16, 21, 26, 31, 36, 48, 72, 96, 120 and 144h after inoculation time) using a pressure transducer (Delta Ohm DTP704-2BGI, Herter Instruments SL, Barcelona). At the end of the incubation (after 144 h), the contents of each serum bottle were filtered using sintered glass crucibles (coarse porosity no. 1, pore size 100–160 µm) under vacuum. Then, the residue was washed out with a neutral detergent solution at 100ºC during 1 h and oven-dried at 100ºC for 48 h to estimate the potential DM disappearance after 144 h of incubation (D144, g g^{-1} DM) and true disappearance after 144 h of incubation (TD144, g g^{-1} DM). Incubations were performed using three different inocula (rumen fluid from three sheep used separately) with two bottles per rumen fluid inoculum (for a total of six observations –three replicates– per sample). In order to estimate the fermentation kinetic parameters, gas production data were fitted using the exponential model proposed by France *et al*. (2000):

$$G = A\left[1-\mathrm{e}^{-c(t-L)}\right] \text{for } t \geq L,$$

where G (ml g^{-1}) denotes the cumulative gas production at time t; A (ml g^{-1}) is the asymptotic gas production; c (h^{-1}) is the fractional rate of substrate fermentation and L (h) is the lag time. Volume of gas (ml g^{-1}DM) produced after 24 h of incubation (G24) was used as an index of digestibility and energy feed value as suggested by Menke and Steingass (1988). According to France *et al*. (2000), the extent of degradation in the rumen (ED, g g^{-1}DM) for a given rate of passage (k, h^{-1}) was estimated as

$$ED = \frac{c \times D144}{c+k}\mathrm{e}^{-kL},$$

To calculate ED, a rate of passage of 0.03 h^{-1} (characteristic for sheep fed with forage diet at maintenance level) was used.

Polyethelenglycol (PEG) bio-assay for the assessment of tannins

The gas production technique described above was also used for this biological assay. Incubations were carried out in serum bottles with or without the addition of 500 mg PEG. Ground samples (300 mg) were weighed out into serum bottles, kept at approximately 39ºC and flushed with CO_2 before use. Two bottles were used for each substrate with each inoculum source (rumen fluid from three sheep was used separately as three different inocula giving three replicates per treatment), one for each treatment (with or without PEG). Bottles were tightly closed and placed in the incubator at 39ºC, being shaken at regular times. The volume of gas produced in each bottle was recorded at 6, 12, 24 and 48 h after inoculation time using a pressure transducer. Gas production was corrected by subtracting the volume of gas produced from blank cultures. Tannin activity was calculated as the ratio between cumulative gas measured in the PEG bottle and that recorded in the control (no PEG) bottle for each sample and inoculum. For each sample, values

from the three replicates (inoculum sources) were averaged.

In situ degradability

The procedure to measure *in situ* disappearance has been described in detail by Lopez *et al*. (1991, 1999). *In situ* DM degradability in the rumen of each browse species was determined as the DM disappearance when samples (3 g DM) weighed in nylon bags (45 µm pore size and 7.5 x 15 cm size) were incubated for 24 and 96 h in the rumen of three fistulated Merino sheep fed with alfalfa hay (3 bags per sample and incubation time, one in each sheep). At the end of incubation, bags were removed from the rumen, rinsed with cold tap water and washed in a washing machine with cold water for 3 cycles of 3 min each. The washed bags were dried in a forced draft oven at 100ºC for 48 h, and the residual DM used to calculate *in situ* DM disappearance (IDDM) at each incubation time. After 48 h, the CP and NDF concentrations in the residues were also measured to determine the *in situ* disappearance of crude protein (IDCP) and *in situ* disappearance of NDF (IDNDF). Two bags per sample were washed following the same procedure without being previously incubated in the rumen to estimate DM disappearance at 0 h (estimate of DM solubility and particle loss from the bag).

Statistical analysis

One-way analysis of variance (Steel and Torrie, 1980) was performed on *in vitro* digestibility, gas production fermentation kinetics and *in situ* degradability data with browse species as the only source of variation as factor A and source of inoculum as factor B. Tukey's multiple comparison test was used to determine which means differed from the rest ($p<0.05$). Analysis of variance and correlation analysis were performed using the SAS software package (SAS Institute, 2008), respectively.

Results

The forage used in the present study varied substantially in chemical composition (Table 1). The crude protein content was particularly high for vetch-oat hay (112 g kg^{-1} DM) and the lowest for pedicels (33 g kg^{-1} DM). The cell wall content ranged from 536 to 629 g NDF/DM and from 317 to 447 g ADF/DM. The ADF: NDF ratio was higher in palm leaves (0.71) and pedicels (0.62) than vetch oat-hay (0.54). Ash content was relatively high (>100 g/DM) in palm leaves and low for pedicels and vetch-oat hay.

The highest lignin content was recorded for palm leaves (8.6%) while vetch-oat hay showed the least value (4.5%). This result indicates the high cellulose and/or lignin content of palm leaves and pedicels although there were no significant differences among species in terms of DM and OM.

Table 1. Chemical composition (g kg^{-1} dry matter) of substrates

Plant species	DM (g kg^{-1})	OM	NDF	ADF	ADL	CP	Ash
Palm leaves	955	851	629	447	85.9	60	149.2
Pedicel	939	940	536	335	80.1	33	59.7
Vetch-oat hay	935	942	585	317	45.2	112	58.2

DM, dry matter; OM, Organic Matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin.

Tannin composition of the plant species is presented in Table 2. The highest contents of total extractable tannins were observed in palm leaves whereas pedicels showed lower concentrations. Shrubs from palm tree were rich in condensed tannins, being the highest for palm leaves (885 g kg^{-1} DM) and the lowest for pedicels (674 g kg^{-1} DM). As expected, tannin concentration was particularly low

for Vetch-Oat hay. Based on the results observed with the PEG bioassay, both plant species had higher tannin biological activity and negligible for the control. Tannin values observed with the different techniques were significantly correlated. FCT and TCT (total condensed tannins) were strongly and positively correlated with tannin biological activity (r=0.99, p=0.015; r =0.99; p=0,004 (n=3) at 6 h incubation, respectively). There was no incubation time effect (p=0.326) or a significant interaction (p=0.081) between incubation time and plant species; thus, effects of PEG on gas production (indicative of the presence of tannins) were similar at all incubation times.

Table 2. Phenolic compounds (g kg^{-1} DM, standard equivalent) and tannin biological activity[a] of substrates

Plant species	Total extractable phenols	Total extractable tannins	Free condensed tannins	total condensed tannins	Tannin biological activity [a] at the incubation times:			
					6 h	12 h	24 h	48 h
Palm leaves	58.9	45.2	763.6	885.4	1.364	1.344	1.279	1.256
Pedicels	35.1	25.6	557.3	673.7	1.261	1.138	1.060	1.045
Vetch-oat hay	5.2	1.6	41.1	61.4	0.973	0.985	0.995	1.002

[a]Tannin biological activity as the ratio between gas production measured at different incubation times adding PEG vs. control (i.e., Gas PEG / Gas control).

The samples used in the present study varied substantially in *in vitro* digestibility (Table 3). *In vitro* parameters digestibility (ivDMloss, TIVD and IVD-TT) of the selected species ranged from 0.23 to 0.48; 0.43 to 0.59 and 0.46 to 0.58, respectively (Table 3). As expected, all *In vitro* parameters digestibility of the control were significantly higher than those for palm leaves and pedicels ($p<0.05$).

Table 3. *In vitro* dry matter (g g^{-1} DM) digestibility of substrates and *in vitro* fermentation kinetics (estimated from gas production curves) of substrates

Plant species	ivDMloss	TIVD	IVD-TT	D144 (g/gDM)	DT144 (g/gDM)	G24 (ml/gDM)	A	C	ED
Palm leaves	0.225c	0.433c	0.457b	0.572b	0.618b	61.7c	109.33c	0.0466b	0.141c
Pedicel	0.445b	0.511b	0.560a	0.584b	0.607b	76.0b	192.17b	0.0544a	0.388a
Vetch-oat hay	0.477a	0.599a	0.576a	0.697a	0.722a	165.7a	278.9a	0.0386c	0.361b
S.E.M.	0.009	0.013	0.011	0.021	0.02	12.86	12.8	0.005	0.021

ivDMloss: *in vitro* dry matter loss; TIVD: true *in vitro* digestibility; IVD-TT: *in vitro* digestibility of Tilley & Terry; D144: dry matter disappearance after 144 h of incubation; DT144: true DM disappearance after 144 h of incubation; A: asymptotic gas production; G24: gas production at 24 h of incubation; c: Fractional rate of fermentation; ED: extent of degradation for a fractional passage rate of 0.03 h^{-1}; S.E.M: standard error of the mean; S.E.M: standard error of the mean; [a, b, c, d, e, f, g] Means of column with different letters are significantly different ($p<0.05$).

Data of *in vitro* fermentation kinetics are shown in Table 3. The lowest values of gas production, D144 and ED were observed for palm leaves followed by pedicels whereas vetch-oat hay had significantly higher values. Similar trends were observed for the *in vitro* fermentation kinetics estimated from the gas production curves.

The extent of degradation (ED) of investigated browses ranged between 0.14 g g^{-1} DM for palm leaves and 0.39 g g^{-1} DM for the pedicels. Unexpected D144 value (0.58 g g^{-1} DM) was also observed in pedicels while vetch-oat hay revealed the highest values (0.69 g g^{-1} DM). Similar trends were observed for asymptotic gas production (A) and G24. The rate of gas production (*c*) was the fastest one in pedicels and the slowest for the control.

The highest asymptotic gas production was observed in the control (278 ml g^{-1}

DM) whereas palm leaves recorded the lowest value (109 ml g^{-1} DM).

In situ DM, NDF and CP disappearance coefficients are shown in Table 4. After 96-hour incubation time, vetch Oat hay shows the highest values whereas palm leaves recorded the lowest values. TEP and TET were strongly and negatively correlated with IDDM (r =-0.99, p=0.016; r =-0.99; p=0.019 (n=3) at 6 h incubation, respectively) suggesting that these *in vitro* techniques can be appropriate for detecting the presence of anti-nutritional substances in shrubs.

Table 4. *In situ* disappearance of dry matter (IDDM), *In situ* disappearance of crude protein (IDCP) and *In situ* disappearance of NDF (IDNDF) (g g^{-1} incubated) at different incubation times of substrates

Plante species	*In situ* DM disappearance after incubation times:			*In situ* NDF disappearance after incubation times:			*In situ* CP disappearance after incubation times:		
	0 h	24 h	96 h	0 h	24 h	96 h	0 h	24 h	96 h
Palm leaves	0.206c	0.359b	0.456b	0.010b	0.177a	0.257b	0.247c	0.418c	0.541b
Pedicels	0.379a	0.460a	0.523a	0.128a	0.144b	0.183c	0.421b	0.517b	0.548b
Vetch-Oat hay	0.322b	0.466a	0.540a	0.133a	0.189a	0.603a	0.789a	0.803a	0.825a
S.E.M	1.133	2.366	2.854	0.196	2.671	0.196	0.010	0.014	0.011

S.E.M: standard error of the mean; a, b, c, d, e, f, g Means in a column with different superscripts are significantly different (p<0.05).

Kinetic parameters of *in situ* disappearance IDDM, IDCP and IDNDF of substrates are shown in table 5. IDDM showed the highest asymptotic gas parameter (A), rate and extent of rumen degradation for Vetch-Oat hay whereas Pedicels observed almost similar values. Considering the unexpected results obtained for pedicles in terms of gas production and *in vitro* digestibility, this fraction of date palm can be considered as a potential feed nutriment for animal nutrition.

Table 5. Kinetic parameters of *in situ* disappearance of dry matter (IDDM), *in situ* disappearance crude protein (IDCP) and *in situ* disappearance NDF (IDNDF) of substrates

Plant species	*In situ* DM disappearance			*In situ* NDF disappearance			*In situ* CP disappearance		
	A	*c*	ED	A	*c*	ED	A	*c*	ED
Palm leaves	0.443b	0.074a	0.315b	0.257b	0.200a	0.285b	0.542b	0.061a	0.379c
Pedicels	0.523a	0.0884a	0.390a	0.181c	0.200a	0.313a	0.548b	0.125c	0.464b
Vetch-Oat	0.540a	0.0823a	0.395a	0.627a	0.04b	0.252c	0.825a	0.159b	0.579a
S.E.M	0.0152	0.0153	0.0154	0.0063	0.0142	0.0170	0.093	0.0192	0.0145

A (g/100g DM): asymptotic gas production, c(h^{-1}): fractional rate of fermentation; ED(g/g DM): extent of degradation for a fractional passage rate of 0.03 h^{-1}; S.E.M: standard error of the mean,a, b, c, d, e, f, g :Means in a column with different superscripts are significantly different (p<0.05).

Discussion

The arid regions are represented in part by oasis where the cultivation of date palm trees is preponderant. Local farmers use date palm by products, principally discarded dates, leaves and racemes for ruminant feeding supplementation (Genin*et al*., 2004). Although those date palm fractions gain increasing significance as the nutritional value of grass drops, they never reach a prominent place in the diet because of their low CP content, high fibre or tannin contents or low digestibility.

An interesting challenge for scientists in the field of animal nutrition is the introduction of alternative feedstuffs that could surmount the problems of environmental harshness and production costs. Some indigenous browse species are useful sources of animal feeds and can provide green forage for animals at times when grass and herbaceous species are of low nutritional value.

Chemical composition of palm leaves observed in this study was similar to that reported by Pascual*et al*. (2000), but the NDF was lower than that mentioned by

Genin*et al*. (2004). These differences could be due to the characteristics of the soil type, the age of the date palm trees, the date palm tree variety and environmental conditions on the nutritional proprieties of shrubs. The high level of fiber content in palm leaves and pedicels could be explained partly by the ecological conditions-high temperature and low precipitations tend to augment the cell wall fraction and to decrease the soluble contents of the plants (Pascual*et al*., 2000). Similar results had been reported by Boufennara*et al*. (2012) who found a high level of fiber content (NDF, ADF and lignin) for Degletnour palm tree variety. These similarities of results are probably due to the fact that these samples are collected from almost the same arid climatic environment and probably with the same soil type. Lignin fraction is considered an indigestible fraction and inhibits the access of microbial enzymes to the structural polysaccharides of the cell wall (Van Soest, 1994).

In semi-arid regions and in the dry season, crude protein content of the herbaceous rangeland vegetation decreases significantly which induces a prolonged period of under-nutrition of livestock's (Yayneshet*et al*., 2009). In developing countries where food resources for food and feed are deficient, only low quality forages, crop residues and agro-industrial by-products available are used for feeding ruminants. Under these conditions, the use of supplementation is inevitable and essential (Deffairi and Arhab, 2016).

The CP content of the investigated browses studied herein was lower than the minimum level of 7-8% DM required for optimum rumen function and feed intake in ruminant livestock (Van Soest, 1994; Boufennara *et al*., 2012; Bouazza *et al*., 2012). Norton *et al*. (1994) affirmed that this type of feeds cannot supply the minimum amount of nitrogen necessary to ensure an optimal metabolic activity of ruminal microbiota. In the present study, CP content was particularly low (<100 g/DM) in palm leaves in agreement with data reported for other Tunisian and Mediterranean shrubs (Cabiddu *et al*., 2000; Frutos *et al*., 2002). Deffairi and Arhab (2016) reported low nitrogen content (6%) in palm leaves.

However, the presence of anti-nutritional secondary compounds (e.g. polyphenols, tannins) with potential adverse effects on rumen microbial fermentation, feed digestibility and animal performance could restrict nutrient utilization of shrubby vegetation (Waghorn and McNabb, 2003). Plant secondary compounds had antimicrobial effects by acting against bacteria, protozoa and fungi and are the main active components (Burt, 2004). The use of different analytical methods can lead to large variations in the final tannin results (Makkar, 2003). Palm leaves and pedicels can be considered tanniniferous plants because of their high CT compared with the control.

Due to their phenolic compounds which are beneficial in many applications in animal nutrition plants with antioxidant properties have received special research attention mainly (Atawodi *et al*., 2013). However, African tropical browses have been shown to contain varying quantities of condensed tannin and other anti-nutritional substances in their biomass that affect their optional utilization by animals.

It is noteworthy that some of the CT concentrations measured cannot be considered realistic. For instance, it is not possible that the CT content in palm leaves foliage is over 700 g kg^{-1}DM. It seems also very unlikely that CT concentration is to be higher than TEP concentration as there are many other phenolic compounds different from condensed tannins. These improbable results can be due to deficiencies and weakness in the butanol-HCl technique to

measure condensed tannins (GSchofield *et al.*, 2001). The major deficiency of this method is the lack of a reliable and authentic standard to estimate CT concentrations from the absorbance values (Ammar *et al*., 2005). Purified quebracho tannin has been questioned as standard because of its globular structure and the small number of hydroxyl groups that are responsible for a low reactivity of the molecule, resulting in weak absorbance values even with high concentrations of quebracho tannin (Schofield *et al*., 2001), thus overestimating the concentrations of CT in the samples when calculated from the standard curve obtained with quebracho tannin. Makkar (2000) has suggested to use only the absorbance values or to apply a conversion factor to estimate CT concentrations as leucocyanidin equivalent from absorbance values. Although this may give values that could be considered more realistic, few authors have raised the possibility that the reaction with the butanol-HCl-iron reagents may be specific for each specific tannin depending on the number and type of reactive groups present in its molecule. Thus, the butanol-HCl reaction should be used with caution as a quantitative assay due to the heterogeneity of CT and the lack of appropriate standards for their quantification (Ammar *et al*., 2005).

Digestibility of the legume browse samples was determined by two conventional and extensively *in vitro* techniques (Tilley and Terry, 1963; Van Soest *et al*., 1966). The potential degradability and degradation rates were estimated from gas production profiles derived from measurement of fermentation gas produced when the sample plant was incubated *in vitro* in diluted rumen fluid (Theodorou *et al*., 1994). This method has been accepted as a trusty tool in feed evaluation because gas production is well correlated with microbial protein synthesis (Blümmel*et al*., 1997), and *in vivo* and *in vitro* digestibility (Khazaal *et al*., 1993). With the data reported herein, gas production parameters (ED, G24 and c) were positively correlated (r-values ranging from 0.666 to 0.939; $p<0.001$) with *in vitro* digestibility values obtained with both gravimetric techniques.

The biological activity of the microbiote ruminal measured through the specific effects of tannins by the use of the PEG shows that tannins influence the production of gas in various degrees. Besides, the obtained results for vetch-oat hay show that the addition of the PEG has almost no significant effect on the production of gas and confirms it slow contents in TCT.

Variations in the results of *in vitro* dry matter digestibility can be attributed to several factors such as processing of samples, difference in chemical composition or handling of equipment (Adel *et al*., 2017). Furthermore, The CP, fiber contents, DM degradability and IVOMD values are used as indicator to use as feed supplements for ruminant (Andualem *et al*., 2016). In addition, Foguekem *et al*. (2011) suggested that *in vitro* dry matter digestibility is strongly influenced by the amount of fiber represented by the NDF, ADF and cellulose levels in the plant tissues.

Bacterial CP contamination of the *in situ* bag residues may have been partly responsible for the generally low measured digestion in the rumen (Gasmi-Boubaker *et al*., 2005). Despite the process of washing bags, residues can still contain substantial quantities of microbial matter and the highest contamination is usually observed in fibrous feeds (Bernard *et al*., 1988). The low degradation for palm leaves may also be due to the influence of the tannins, which inactivate digestive tract enzymes, and reduce protein availability resulting in low degradation (Melaka *et al*., 2003). It has been shown that *in situ* CP degradability is negatively correlated to

phenolic compound concentrations (Melaka *et al*., 2003).

Mertens (1993) indicated that the factors of a physical nature such as crystallinity and degree of polymerization of the polysaccharides of the cell walls can have a significant effect on the kinetics of degradation as well as lignin content. The specific examination of the kinetic data of the studied substrates reveals that the *in situ* NDF fraction degradation occurs mainly between 24 and 96 hours.

The addition of the PEG engenders an increase of the bacterial biomass for the substrate rich in tannins according to the nature of feeds and their chemical composition in terms of phenolic compounds. This study confirms that the use of the PEG combined to the technique of gas production and the *in vitro* digestibility and *in situ* essays can be a simple and fast way for the preliminary classification of all kinds of plants species without passing by the boring and expensive zootechnic studies.

The fraction of crude protein and tannins compounds does not seem to play a significant rolein the process of *in situ* degradation of the cell wall. The potential effect of phenolic compound on ruminal fermentation is poorly detected by *in sacco* method (Apori *et al*., 1998). Indeed, the effect of the anti-nutritive factors which are unlikely to be detected using *in sacco* method could account for the differences between the two methods. In the *in vitro* gas production technique which is a batch system with limited supply of rumen fluid, these anti-nutritive factors remain in the fermentation medium and affect rumen microbial activity (Boufennara *et al*., 2014). Conversely, in the *in sacco* technique which is an open system with real rumen environment with a continuous microbial activity and growth, the inhibition would be transient. Khazaal *et al*. (1993) reported that the technique of *in vitro* gas production is more sensitive than *in sacco* technique for determining the nutritive value of forages containing tannins.

Conclusion

Combined use of chemical analysis, an *in vitro* gas production and *in situ* incubation technique are advocated to determine the nutritive value of feeds containing phenolic compounds. On the basis of these techniques, pedicels have better nutritive potential than palm leaves offering considerable potential as good forage for ruminants during critical periods in the semi-arid regions of Algeria. The negative correlation between tannins compounds and digestibility suggests that the *in vitro* techniques can be appropriate for detecting the presence of anti-nutritional substances in shrubs.

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مقایسه ترکیبات شیمیایی و هضم پذیری ساقه و برگ نخل به عنوان منبع تغذیه دام با استفاده از روش درون تنی و برون تنی

سوهیل بوفنارا[الفج*]، لیاس بوازا [الف]، سمیر مدجکال [ب]، خالد رحال [الف]،ایوان ماتسو [ج]، ماریا جوسه رانیلا [ج]، سکاندینو لوپز[ج]
[الف]گروه سلولی مولکولی، دانشکده علوم طبیعی، دانشگاه آبس لاغور خنچال، الجزایر، [*](نگارنده مسئول)، پست الکترونیک: bouffenara@yahoo.fr
[ب]گروه بیوشیمی و میکروبیولوژی، دانشکده علوم، دانشگاه مد بودیاف ماسیلا، الجزایر
[ج]موسسه گانادریا د مونتانا، دانشکده تولیدات دامی، اسپانیا

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چکیده: هدف از این تحقیق مشخص کردن ترکیب شیمیایی و هضم‌پذیری و برآورد مولفه‌های تخمیر در برگ‌ها و ساقه درخت خرما به روش درون تنی است. همچنین برای بررسی ماده خشک، پروتئین و فیبر قابل حل قابل هضم و سرعت و میزان تولید گاز، روش برون تنی مورد استفاده قرار گرفت. یونجه ویت چاودار به عنوان ماده غذایی مرجع در نظر گرفته شد. نمونه‌ها در منطقه تولگا(جنوب شرقی الجزایر) گرفته شد. نتایج نشان داد که محتوای پروتئین خام گونه‌های گیاهی در ساقه و برگ‌های نخل کم بود(به ترتیب ۳۳ و ۶۰ گرم در کیلوگرم ماده خشک). بیشترین مقدار فنل‌های قابل استخراج، تانن و تانن‌های فشرده شده در برگ‌های نخل مشاهده شد در حالی که ساقه نخل غلظت کمتری را نشان می‌داد. هضم پذیری به روش درون تنی و حذف ماده خشک به روش برون تنی برای علوفه‌های مورد آزمایش، کمی متفاوت بود. روش‌های مشابهی برای تخمین سینیتیک تخمیر به روش درون تنی از روی منحنی تولید گاز، مشاهده شده است. ساقه‌ها بیشترین میزان تجزیه‌پذیری را نشان دادند در حالی که به نظر می‌رسد برگ‌های نخل، مواد با تجزیه‌پذیری کمتر به ترتیب با ضریب ۰/۳۹ و $0/14 g g^{-1}$ را دارند. باوجود پروتئین خام متوسط و محتوی فیبر بالا همراه با هضم‌پذیری و حذف شدن ماده خشک موجود در ساقه‌ها، این گیاه در مقایسه با یونجه ویت چاودار می‌تواند ارزش غذایی بیشتری داشته باشد. حذف شدن ماده خشک پس از ۱۴۴ ساعت انکوباسیون، با ترکیبات فنلی و مجموع تانن‌های قابل استخراج رابطه منفی داشت و این نشان می‌دهد که روش درون تنی می‌تواند برای تشخیص وجود مواد بدون ارزش غذایی در بوته‌ها مناسب باشد.

کلمات کلیدی: ترکیبات شیمیایی، هضم‌پذیری درون تنی، روش رون تنی، تانن‌ها، درخت خرما