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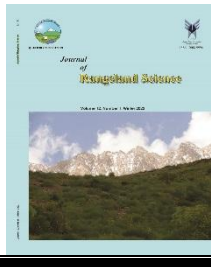

Research and Full Length Article:

In vitro Gas Production and Fermentation Parameters of Some Plants Species Collected from Algerian Arid Rangelands

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Abstract. The objective of the study was to evaluate the nutritional value of some native plants, collected from Algerian arid zones by *in vitro* gas production method. The volatile fatty acids (VFA) of samples were also determined in the culture medium. This work was carried out in 2011 and was conducted in comparison to a control substrate (oat vetch) considered as a reference plant in literature. The selected plants were collected in the arid areas of the Algerian desert. Nine forages including six dicotyledon plants (*Atriplex halimus* L., *Artemisia campestris* L., *Artemisia herba-alba* subsp. valentina, *Astragalus gombiformis* Pomel, *Spartidium saharae* (Coss. & Dur.) and *Retama raetam* Forssk., and three monocotyledon plants (*Stipagrostis pungens* (Desf.), *Lygeum spartum* L.and *Stipa tenacissima* L.) were selected. *A. campestris, A. gombiformis* and *A. herba-alba* recorded the highest values of gas production. The total VFA production of the different substrates is significantly different between them (p<0.0001). *A. gombiformis* had the highest total VFA (34.7 mmol/L) followed by *A. campestris* (32.8 mmol/L), while the lowest total production of VFA was observed in *S. tenacissima* (17.3 mmol/L). Generally, the plant studied can be classified in two groups, one group with poor-quality grasses (*L. spartum*. and *S. tenacissima*) and other with higher digestibility (A. gombiformis and Artemisia spp.). In conclusion, dicot species are therefore recommended for feeding ruminants.

Key words: Gas Production, Volatile Fatty Acids, Nutritive Value, Forages, Rumen

Introduction

In Algeria, two million square are desert (arid) over a total area of 2381740 km^2 (Nedjraoui, 2001), while the rest (381740 km^2) is semi-arid (semi-desert) and sub-humid (semi-wet, semi-dry).The different regions of Algeria are classified according to the climatic nature and rate of rainfall.

The nutritional value of feedstuffs is traditionally determined by the content of chemical components, as well as their rate and extent of degradation, which are estimated from the release of fermentation end-products (Getachew *et al.,* 1998; Boufennara 2012; Bouazza *et al.,* 2019). In the last few years there has been a growing interest in livestock production which plays a major role in the national economy through the provision of animal products for local consumption and foreign exchange. The most of the livestock are kept under an extensive management system and are fed exclusively on rangeland forages (Mayouf and Arbouche, 2014). Trees, shrubs and forages are important sources of feed for ruminants, particularly in areas with long dry period and harsh environmental conditions, as in the Mediterranean regions, despite the fact that, their feed quality is not as high as that of herbaceous species (Papanastasis *et al*., 2008). The value of these plants in animal nutrition is associated with features such as their abundant supply. Additionally, in arid areas, the majority of these plants are widespread where livestock production is a crucial source for farmers' income, in contrast to annual plants that generally have shorter growing periods and lower yields (Gokkuş and Koc, 2001). Although these resources gain increasing significance as the nutritional value of grass drops, they never play remarkable role in animal diet. This fact is caused by the low crude protein (CP) content and high fibre contents and low digestibility of theses local forages (Cabiddu *et al*., 2000). In this context, studies on these plants collected in the arid zones have been conducted by some researchers (Boufennara *et al.*, 2012; Arhab, 2006; Larbi *et al.*, 1998; Medjekal *et al.*, 2018).

In literature, the most valuable methods for determining nutritional value are: *in vitro* digestibility technique (Tilley and Terry, 1963), *in situ* degradation technique (Orskov and McDonald 1979) and *in vitro* gas production technique (Menke and Steingass 1988). It is pertinent to note that biological methods are much more significant and give very good results compared to conventional chemical and physical methods. Indeed, microorganisms and enzymes are more sensitive to factors influencing the rate and extent of digestion (Ballet 1989).

The association between ruminal fermentation and gas production has long been known (Getachew *et al*., 1998). They are currently considered as routine techniques in the assessment of the nutritional value of foods following the work of Menk *et al*. (1979) in which a strong correlation between *in vitro* gas production and apparent digestibility has been established.

This study was conducted with objective to evaluate various browse species collected from a semi-arid zone in Algeria based on the determination of their gas production and VFA concentration in the edible part of the plants, considered as useful indicators for the preliminary evaluation of some feeding resources and to select the most nutritionally interesting forage plants.

Materials and methods

The area of study

This experiment was conducted in Bousaâda district, north central Algeria (35° 15.768' N, 04° 13.885' E). This region is situated in the Saharan Atlas region, at the northern edge of the Sahara Desert between the Atlas Mountains and the El-Hodna depression and salt lake. According to the Köppen classification, the climate of this region is BWh (dry desert climate), characterized by high temperatures ranging between 24 and 41°C, and scarce and erratic annual

precipitations for a total of 350-700 mm. Under these environmental conditions, the plant species studied show a slow vegetative growth and phenological development throughout most of the year, often lagged in response to the infrequent major rainfalls.

Plant species

Nine browse plant species were used in this study: six dicotyledon plants namely (*Atriplex halimus*, *Artemisia campestris*, *Artemisia herba-alba*, *Astragalus gombiformis*, *Spartidium saharae* and *Retama raetam*) and three monocotyledon plants (*Stipagrostis pungens*, *Lygeum spartum* and *Stipa tenacissima*). Samples were collected when plants were at a flowering (*A. halimus, A. gombiformis, R. raetam and L. spartum*) or at a mature stage (the rest of species).

The plants were selected based on their availability for grazing small ruminants and their relative abundance in the studied area. Leaves, thin twigs (young stems) and some flowers (when existing) were clipped with scissors from the aerial part of the plants and taken immediately to the laboratory. Vetch-oat hay was supplied by the technical Institute of Breeding, Ain-Mlila, Algeria. Samples were pooled, oven-dried at 50 ºC (Makkar, 2003) and subsequently ground to pass a 1 mm screen.

Rumen fluid preparation

For the extraction of rumen fluid as inoculum, six mature Merino sheep (body weight 49.4 ± 4.23 kg) fitted with a permanent ruminal fistula were used.

Animals were fed with lucerne hay ad libitum (170 g CP, 499 g NDF, 361 g ADF and 61 g ADL kg^{-1}DM). The sheep's had free access to water and mineral/vitamin block. Ruminal fluids were collected prior to morning feeding, transferred into thermos flasks and moved immediately to the laboratory, where rumen fluid was strained through various layers of cheesecloth and kept at 39°C under a constant flow of CO_2. Then, the rumen fluid was diluted (1:4 v/v) with a culture medium containing macro- and micro-mineral solutions, resazurin and a bicarbonate buffer solution and prepared as described by Menke and Steingass (1988). The medium was kept at 39°C and saturated with CO_2. Oxygen in the medium was reduced by the addition of a solution containing cysteine hydrochloride and Na_2S as described by Van Soest *et al*. (1966).

In vitro gas production

The technique of gas production of Theodorou *et al*. (1994) was used in this experiment. In 120 ml serum bottles, 500 mg of ground samples were incubated in 50 ml of diluted rumen fluid under a CO_2 constant flow. In order to compensate the gas production in the absence of substrate, six bottles containing only diluted rumen fluid were incubated as blanks. All bottles were closed with rubber stoppers, crimped with aluminium seals, shaken and placed in an incubator to controlled temperature 39°C once they are filled up. The amount of gas production was recorded at several incubation times (3, 6, 9, 12, 16, 21, 26, 31, 36, 48, 72, 96, 120 and 144 h after inoculation time) using a pressure transducer (Delta Ohm DTP704- 2BGI, Herter Instruments SL). The equation used to transform gas pressure (PSI) to (ml/) was: **V= 5.41 x (P1-P2)**

Where: P1 is the bottle pressure at t time and P2 is the blank bottle pressure at t time (Lopez *et al*., 2007).

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).

Volatile fatty acids

In vitro fermentation end-products were assessed in 24 h batch cultures. Samples (400 mg) of each substrate were incubated in serum bottles filled with 40 ml of buffered rumen fluid at 39 °C. After 24 h of incubation, Bottles were opened, pH

was measured and a sample (0.8 ml) was added to a deproteinizing solution (0.5 ml) for volatile fatty acids analysis. Volatile fatty acids were determined by gas chromatography using flame ionization detector and following the technique of Jouany (1982) in an Agilent 6890 apparatus (Agilent Technologies España S. L., Madrid, Spain). The carrier gas pressure was set at 0,5 bar. The carrier gas pressure was set at 0.5 bar. The temperatures of injector, detector and column furnace were 220°C, 250°C and 230°C respectively. The internal standard method (Crotonic Acid) was used to calculate the concentrations of VFA (Garcia-Gonzalez *et al*. 2008).

Statistical analysis

One-way analysis of variance (Steel and Torrie, 1980) was performed on *in vitro* gas production fermentation kinetics and fermentation parameters, with browse species as the only source of variation (fixed effect) and source of inoculum (random effect) as a blocking factor. Tukey's multiple comparison test was used to determine which means differed from the rest (p<0.05). Pearson linear correlation coefficients were determined pair-wise between the variables studied. The software package SAS (SAS Institute, 2008) was used for analysis of variance and correlation.

Results

In vitro gas production

Data of chemical analysis (table 1) of the plants studied were published by Boufennara *et al*. (2012). The overall measurement results of gas production are summarized in Table 2. It has been found that *A. campestris*, *A. gombiformis* and *A. herba-alba* are the plants most fermented by the ruminal microorganisms, until the 36 h of incubation. After 144 h of incubation, oat vetch takes over and is more degradable than all other substrates, producing the highest volume of gas (283 ml/g DM). This situation is essentially related, respectively, to the differences in their soluble (nitrogenous fraction) and insoluble contents (NDF, ADF and ADL fractions). Differences are also noted between the same family species (legume). *L. spartum* and *R. raetam* record very appreciable gas production volumes.

Table1. Chemical composition (g/kg DM**)** and phenolic compounds (g/kg DM, standard equivalent) of the selected plants*

Plants	DM (g/kg)	OM	NDF	ADF	ADL	CP	Ash	TEP	TET	TFC	TCT
A. halimus	932.2	804.46	360.1	181.3	59.9	153.6	195.5	16.1	8.40	39.3	66.3
A. campestris	986.9	897.61	330.4	211.5	97.5	115.0	102.4	84.3	57.1	62.7	114.3
A. herba-alba	951.2	920.00	378.1	258.4	101.1	123.9	80.0	63.7	36.4	80.6	118.8
A. gombiformis	945.4	870.83	340.4	218.0	46.7	223.4	129.2	13.6	3.04	51.6	78.3
C. saharae	946.5	954.49	573.9	427.2	135.2	109.8	45.5	29.9	9.61	76.4	109.7
R. raetam	947.6	956.09	623.2	445.4	199.5	108.7	43.9	8.52	2.03	40.5	77.9
S. pungens	946.0	945.42	770.8	424.9	58.3	95.2	54.6	10.2	4.8	46.6	78.7
L. spartum	948.2	935.49	800.5	535.2	62.5	72.7	64.5	35.6	11.1	77.2	102.4
S. tenacissima	931.0	964.12	792.6	475.5	73.2	74.6	35.9	12.2	3.9	165.5	213.9
Vetch-oat	935.1	941.76	585.2	316.8	45.2	112.4	58.2	5.21	1.62	41.0	61.4

ADF: acid detergent fiber; ADL: acid detergent lignin; CP: crude protein; DM: dry matter; NDF: neutral detergent fiber; OM: Organic matter; TEP: total extractable phenols; TET: total extractable tannins, TFC: Free condensed tannins; TCT: total condensed tannins. * Data published (Boufennara *et al.*, 2012).

Table 2. Cumulative gas production(ml/g DM) of the selected plants in the culture medium

Plants	3h	6h	9h	12h	16h	21h	26h	36h	48h	72h	120h	144h
A. halimus	14.4^{e}	31.0^{ef}	48.7^{d}	66.3^{d}	89.4^{e}	107^{e}	129^{e}	145^{f}	157^{ef}	169^{d}	180^{e}	185^{d}
A. campestris	32.7^{ab}	70.4^{a}	107^{a}	35.1^{a}	162^{a}	185^{a}	203^{a}	219^{a}	228^{a}	232^{b}	238^{c}	239^{b}
A. herba-alba	30.5^{bc}	66.2^{ab}	98.4^{a}	25.3^{a}	153^{ab}	174^{ab}	190^{ab}	201^{ab}	206^{ab}	211^{c}	215^{d}	216^{c}
A. gombiformis	37.4^{a}	76.4^{a}	107.9^{a}	25.9^{a}	147^{b}	164^{b}	178^{bc}	194^{bcd}	201^{b}	208^{c}	215^{d}	217^{c}
S. saharae	26.5^{bcd}	52.2^{cd}	75.5^{b}	95.0^{b}	112^{cd}	128^{cd}	148^{d}	179^{cde}	195^{b}	206^{c}	213^{d}	214^{c}
R. raetam	22.6^{d}	44.0^{de}	63.2^{c}	80.2^{c}	97.7^{de}	119de	142de	175de	193^{bc}	212^{c}	225^{c}	228^{bc}
S. pungens	8.53^{f}	16.4^{g}	23.6^{f}	29.6^{f}	39.9^{f}	56.0^{f}	77.5^{fg}	121^{g}	163^{d}	211^{c}	258^{b}	269^{a}
L. spartum	9.82^{e}	17.7^{g}	26.5^{ef}	34.4^{e}	46.1^{f}	63.0^{f}	81.8^{f}	113^{g}	139^{e}	179^{d}	226^{cd}	242^{b}
S. tenacissima	25.7^{cd}	57.6^{bc}	79.1^{b}	94.7^{b}	111^{dc}	128^{cd}	143de	161^{ef}	174^{cd}	184^{d}	194^{e}	198^{d}
Vetch-oat	25.7^{cd}	51.9^{dc}	74.9^{b}	95.2^{b}	116^{c}	139^{c}	161^{cd}	197^{bc}	225^{a}	257^{a}	278^{a}	283^{a}
SEM	5.24	8.11	8.74	9.98	11.7	14.5	16.7	17.1	16.0	12.4	12.1	12.4

h: hour; SEM: 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).

Relationship between gas production parameters and chemical components

The correlations between gas production and chemical constituents of the substrates studied are shown in Table 3. The NDF fraction is significantly and negatively correlated with gas production for the first stages of fermentation less than 26 h (r =-0.72; p<0.01; T=3h). ADF content is negatively and significantly correlated with gas production before 26 h (r =-0.58, p<0.05; r =-0.68, p<0.05; r =-0.69, p<0.05; r =- 0.73, p<0.01; r =-0.71, p<0.01), respectively for V3, V6, V9, V21, V26. This can be explained by the fact that the effect of the degradation of the NDF and ADF wall fractions on the digestibility process increases and then decreases over time as this fraction is gradually degraded. After 26 h of fermentation, the correlation coefficient is weak and insignificant (p>0.05), which proves that most of these fractions are degraded and does not play any role in the digestibility process.

Table 3. Correlation coefficients between chemical composition parameters (g/kg DM), volume of *in vitro* gas production (ml/g DM) and kinetics parameters of the selected plants

In vitro Gas Production	NDF	ADF	ADL	CP	TT	FCT	ACT	TCT	A	*c*
V3	-0.72**	-0.58*	0.21	0.50	0.15	-0.23	-0.12	-0.21	0.08	0.60*
V6	-0.80**	-0.68*	0.16	0.47	0.28	-0.18	-0.05	-0.16	0.10	0.62*
V9	-0.82**	-0.69*	0.17	0.47	0.32	-0.20	-0.07	-0.19	0.11	0.64*
V21	-0.84***	-0.73**	0.20	0.45	0.31	-0.31	-0.18	-0.29	0.22	0.62*
V26	-0.81**	-0.71**	0.23	0.44	0.26	-0.36	-0.23	-0.35	0.28	0.57
V48	-0.58	-0.55	0.21	0.34	-0.01	-0.57	-0.44	-0.56	0.53	0.29
V72	-0.30	-0.36	0.08	0.22	-0.19	-0.70*	-0.60*	-0.69**	0.66*	0.07
V96	-0.10	-0.21	-0.01	0.12	-0.30	-0.74**	-0.69**	-0.73**	0.70*	-0.09
V144	0.12	-0.03	-0.99**	0.02	-0.38	-0.73**	-0.69*	-0.73**	0.712**	-0.24

*: p< 0.05; **: p<0.01; ***: p<0.001; ns: not significant (p>0.05). A: Asymptotic gas production; ADF: acid detergent fiber; ADL: acid detergent lignin; ATC : attached condensent tanins; *c:* rate of gas production; CP: crude protein; NDF: neutral detergent fiber; TT: total tanins; FCT: free condense tanins; TCT : total condensed tanins; V3: cumulative gas production after3 h; V6: cumulative gas production after 6 h; V9: cumulative gas production after 9 h; V21: cumulative gas production after 21 h; V26: cumulative gas production after 26 h; V48: cumulative gas production after 48 h; V72: cumulative gas production after 72 h; V96: cumulative gas production after 96 h; V144: cumulative gas production after 144 h.

Fermentation of end products

Table 4 summarizes volatile fatty acids production for the plants studied herein. The pH recorded in each batch, after 24 h of incubation, ranged between 6.54 and 6.63 (Table 4). The total VFA production of the different substrates is significantly different between them (p<0.0001). *A. gombiformis* had the highest total VFA (34.7 mmol/L), followed by *A. campestris* (32.8 mmol/L) while the lowest production of VFA is observed in *S. tenacissima* (17.3 mmol/L). *A. herba-alba* had an intermediate value of 24.5 mmol/L almost similar to vetch oat (26.2 mmol/L).

Table 4. Total (mmol/L) volatile fatty acids production, molar proportion (%) and acetate to propionate ratio (A:P) after 24 h of *in vitro* incubation of the selected plants.

Plants	pH	Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate	tVFA	C2/C3
A. halimus	6.59^{abc}	62.4^{c}	20.9^{b}	2.07^{c}	9.70^{c}	3.10^{e}	1.93^{e}	29.0^{c}	2.99^{d}
A. campestris	6.54^{c}	69.1^{a}	15.5^{bc}	1.92^{c}	9.00^{c}	2.89^{f}	1.64^{f}	32.8^{b}	4.47^{a}
A. herba-alba	6.57 ^{abc}	65.8^{b}	15.4^{d}	2.53^{cd}	10.10^{c}	3.92^{c}	2.25^{c}	24.5^{de}	4.27^{b}
A. gombiformis	6.59 ^{abc}	65.0^{b}	18.6^{a}	2.34^{e}	8.70^{e}	3.20^{d}	2.08^{d}	34.7^{a}	3.49^{c}
S. saharae	6.56 ^{abc}	62.1^{b}	19.6^{a}	2.16^{d}	10.9^{bc}	3.21^{e}	2.13^{d}	31.5^{b}	3.17^{d}
R. raetam	6.63^{a}	57.3^{d}	20.6^{c}	2.81^{c}	12.3^{b}	4.52^{bc}	2.50^{bc}	22.8^{e}	2.79^{e}
S. pungens	6.58^{abc}	57.6^{d}	18.9^{d}	3.16^{b}	12.8^{b}	5.26^{ab}	2.30^{c}	19.6^{f}	3.05^{d}
L. spartum	6.59^{abc}	56.7^{d}	22.3^{a}	2.88^{c}	11.1^{bc}	4.54^{b}	2.45^{b}	22.9^{e}	2.54^{f}
S. tenacissima	6.61^{abc}	55.6^{e}	18.0^{e}	3.58^{a}	13.8^{b}	6.26^{a}	2.83^{a}	17.3^{f}	3.09^{d}
Vetch-oat	6.55^{bc}	55.4^{e}	20.9^{b}	2.02^{f}	17.2^{a}	2.75^{f}	1.83^{e}	26.2^{d}	2.65^{ef}
SEM	0.029	0.523	0.195	0.021	0.133	0.035	0.031	0.797	0.084

t VFA: total volatiles fatty acids; C2/C3 : ratio acetate propionate; SEM: 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).

Regarding the production of each VFA, the production of acetate also differentiates two groups: *A. campestris* with 69% and *A. gombiformis* 65%, while *S. tenacissima* recorded the lowest concentration (56%). Overall, Asteraceae and legumes have higher acetate values than grasses. Production of propionate varied considerably (p<0.001) among shrubs. *L. spartum* still in the head with a molar proportion of 22%, followed by the legume *A. Halimus* (21%), while *A. herba-alba* had the lowest molar proportion (15%). The acetate/propionate ratios of the two major VFA are significantly different between substrates (p<0.0001). These ratios ranged from 2.54 to 4.47. Asteraceae and *A. campestris* has the highest ratio value while *L. spartum* recorded the lowest rate.

Relationships between chemical components and VFA

The correlations existing between VFA and chemical analysis of the forages studied are summarized in Table 5. NDF and ADF fraction are negatively and significantly correlated with the acetate/propionate ratio (r =-0.70, p<0.05; r =-0.66, p<0.05) respectively. ADF fraction is negatively and significantly correlated with total VFA (r =-0.58, p<0.05). There is a good match between nitrogen content and acetate production (r =0.81, p<0.001), valeric acid (r =0.60, p<0.05) and total VFA (r =0.68, p<0.05). Free condensed tannins and the total condensed tannins have negative and significant correlative effects with total VFA (r =-0.66, P<0.05; r =-0.67, p<0.05) while total condensed tannins (TCT) are negatively and significantly correlated with acetate and propionate (r =-0.62, p<0.05; r =-0.71, p<0.05) respectively.

Table 5. Correlation coefficients between gas production, chemical analysis and volatiles fatty acids of the selected plants.

	NDF	ADF	ADL	CP	FCT	TCT	V3	V6	V9	V21	*c*
pH	0.13	0.33	0.48	-0.41	0.47	0.47	0.15	0.11	0.09	0.14	0.20
Acetate	-0.55*	-0.64*	-0.24	0.81**	-0.61*	-0.62*	0.39	0.38	0.40	0.44	0.58*
Propionate	-0.14	-0.29	-0.05	0.47	-0.69*	-0.71*	0.04	0.01	0.02	0.11	0.24
IsoButyrate	-0.44	-0.29	0.32	0.54	-0.32	-0.33	0.25	0.25	0.28	0.36	0.57*
Butyrate	0.08	-0.14	-0.39	-0.03	-0.37	-0.38	-0.08	-0.10	-0.09	-0.06	0.05
Isovalerate	-0.27	-0.02	0.42	0.40	-0.03	-0.03	0.07	0.07	0.09	0.14	0.40
Valerate	-0.55	-0.51	0.21	0.60*	-0.46	-0.46	0.24	0.26	0.30	0.41	0.56
tVFA	-0.46	-0.58*	-0.22	0.74**	-0.66*	-0.67*	0.31	0.29	0.31	0.37	0.53
C2/C3	-0.70*	-0.66*	-0.29	0.68*	-0.12	-0.11	0.56*	0.62*	0.63*	0.58*	0.63*

*: p< 0.05; **: p<0.01; ***: p<0.001; ns: not significant (p>0.05).ADF: acid detergent fiber; ADL: acid detergent lignin; tVFA: total volatile fatty acids; CP: crude protein; C2 / C3: acetate-propionate ratio; ns: not significant (P> 0.05);c: rate of gas production; CP: crude protein; NDF: neutral detergent fiber; FCT: free condense tannins; TCT: total condensed tannins; V3: cumulative gas production at 3 h; V6: cumulative gas production at 6 h; V9: cumulative gas production at 9 h; V21: cumulative gas production at 9 p.m.

Discussions

In vitro gas production

The use of the *in vitro* gas production methodology to estimate digestion of feeds is based on the well-established relationship between the feed digestibility and *in vitro* gas production, in combination with the feed chemical composition (Menke and Steingass, 1988). The *in vitro* gas production system aids to better assess nutrient utilization, and its accuracy in describing digestibility in animals has been extensively validated. Since the utilization of roughages is largely dependent upon microbial fermentation within the rumen, description of roughages in terms of their degradation characteristics would provide a useful basis for their evaluation. The *in vitro* techniques are useful tools in initial screening studies to rank the forages according to their nutritive quality.

A significant variation in *in vitro* fermentations kinetics was observed among the samples. In general, the majority of the substrates observe significantly higher gas productions than other plants: 66 mountain fodder (33.7 ml/batch at 24 h) (Andrighetto *et al*., 1992), pasture hay (34.8 ml/ batch at 24 h) (Gulsen *et al*., 2004). These comparisons suggest that these substrates can be effectively used as conventional forages. *In vitro* gas production at different incubation times is significantly different between forages (p<0.0001). The forage classification based on gas production results after 144 h is: Vetch-oat hay >*S. pungens*> *L. spartum*> *A. campestris*> *R. raetam*> *A. gombiformis*> *A. herba-alba* >*S. saharae*>*S. tenacissima*> *A. halimus*.

The relatively low gas production recorded for *A. gombiformis* (217 ml/g DM), despite its very high protein content, confirms the findings of Aregheore *et al*. (2000) and Khazaal *et al*. (1993). Indeed, these authors report that the contribution of total nitrogenous matter to gas production is not a significant influencing factor. The result obtained with *S. pungens* (269 ml/ g DM) could be attributed to its moderate free CP content and/or to the complexation of the ammonia produced by deamination with fermentative carbon dioxide (Krishnamoorthy *et al.*, 1995). The weak fermentation of *S. tenacissima* is essentially linked respectively to their high contents in NDF wall fraction and tannins. However, unexpected results are observed for the leguminous plant *L. spartum*, despite the high levels of wall constituents (Boufennara *et al.*, 2012). This plant shows an appreciable rate of fermentation compared to oat vetch hay. This result dose not corroborates with some authors which mention that the *in vitro* gas production is negatively influenced by the fibrous content of food (Larbi *et al.*, 1998).

Lignin fraction has a very late effect with respect to gas production and revealed to be significant for the 144-hour kinetic point (r =-0.11; p<0.001). This result can be explained by the fact that lignin is hardly digestible and its effect is more pronounced at the end of the fermentation process. These results are consistent with the work of Arhab (2006); Getchew *et al*. (2004) and Larbi *et al.* (1998). Crude protein is not correlated with gas production at all kinetic points. This situation is reported by many authors (Areghoreb, 2000; Khazaal *et al*., 1995; Longuo *et al.*, 1989).

Fermentation of end products

For all substrates, pH values remain above the critical threshold for inhibition (pH=6) of growth and cellulolytic activity of the ruminal microbiota (Hoover, 1986). The results of VFA and acetate/propionate ratios are comparable with other studies (Arhab, 2006; Medjekal *et al.*, 2018), indicating that despite the quantitative differences in VFA production observed between the three studies, their qualitative profile is relatively the same. An acetate/propionate ratio of 3.0-4.1 *in vivo* is reported by many authors (De peters 2000; Brown *et al.*, 2002). It is similar to the ratio of 2.68-4.08 measured *in vitro*

(Bouazza *et al*., 2019; Boufennara *et al.*, 2019) and higher than the ratio of 2.0-4.1 measured *in vitro* (Blümmel *et al.*, 1999; Brown *et al.*, 2002). A high ratio indicates appreciable NFD digestibility of foods (Getachew *et al*., 2004, Blummel *et al.*, 1999; Brown *et al*., 2002). Similar ratios are also obtained from other substrates rich in NDF fraction (Getachew *et al*., 2004). Beuvink and Spoelstra (1992) indicate that the production of acetate is related to the gas production, caused by the degradation of the various components of the food. On the other hand, the formation of propionate would rather be related to the gases released from the buffer system. Thus, the VFA fermentation profile obtained confirms the good *in vitro* fermentability of our substrates by the ruminal microbiota.

The classification of shrubs on the basis of quantitative VFA production is as follows: *A. gombiformis*> *A. campestris*> *S. saharae*> *A. halimus*> Vetch-oat> *A. herba-alba* > *L. spartum*> *R. raetam*> *S. pungens*> *S. tenacissima*.

It should be noted that *in vitro* fermentation of the different substrates also leads to the formation of butyric, isobutyric, valeric and isovaleric acids, but at relatively low concentrations. The reference substrate has a high butyric acid molar proportion (17.2%) while *A. gombiformis* recorded the lowest molar proportion (8.70%). According to McDonald *et al*. (1995), fermentation of foods rich in starch tends to produce more propionate, while fibrous substrates result in high concentrations of acetate. This would explain the predominance of acetate production from our substrates, which have a rich NDF content. The gas production mainly accompanies the fermentation pathways producing acetate and butyrate, on but the yield of propionate is due only to the acidic buffer of the medium (Wolin 1975). The strong correlations recorded between total nitrogen matter, valeric acid and total VFA are contrary to the work of Getachew *et al*. (2002). The work of Getachew *et al* (2004) agrees perfectly with the results of the correlation of gas production rate with acetate/propionate ratio and isobutyrate.

The strong correlations between crude protein, valeric acid and total VFA indicate that the fermentation of nitrogen matter contributes to the production of fats volatile acids. Concerning the correlation between the parameters of gas production and the chemical components is explained by the fact that the effect of the degradation of the NDF wall fraction on the digestibility process increases, then decreases over time because this fraction is gradually degraded. However, the lack of correlation after 26 h proves that most of this fraction is degraded and plays any role in the digestibility process. Also, the negative correlation of gas production with ADF content is explained by the point that lignin is difficult to digest and its effect is the more pronounced.

Conclusion

All the chemical and *in vitro* measurements are useful tools in initial screening studies to rank the forages according to their nutritive quality. The results obtained in this study clustered the examined species in two groups according to their nutritive value (considering jointly all the measures of gas production and volatile fatty acids): one including the poor-quality grasses and another one representing the most digestible plants (*A. gombiformis* and *Artemisia* spp.). These dicot species are therefore recommended for feeding ruminants. *S. saharae* and *R. Raetam*, with an interesting quantity of gas production, occupy an intermediate position between the two groups and can be considered as a good nutritional forage.

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