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Study of associative effects of date palm leaves mixed with *Aristida pungens* and *Astragalus gombiformis* on the aptitudes of ruminal microbiota in small ruminants

Deffairi Djamila* and Arhab Rabah

Department of Food Sciences, Blida University, Algeria.

The objectives of this trial were to evaluate interactions between microbial degradation of three substrates: date palm leaves as tannin-rich substrate, *Astragalus gombiformis* (as nitrogen source) and *Aristida pungens* (as carbon source). The model included the principal effects of single substrate and mixtures of multiple interactions. The forages were fermented alone or mixed with date palm leaves in various combinations (0, 10, 20, 30, 40 and 50%), in buffered rumen fluid using *in vitro* gas techniques. Gas production (mmol/g of dry matter) at 3, 6, 9, 24, 48 and 72 h of incubation was measured from all feed combinations. Treatments were carried out in triplicate and data were submitted to analysis of variance using a mixed procedure with repetition considered as the random effect. The interactions between feeds were evaluated on the basis of differences between single and mixed substrates on the different variables measured. The results showed that the included palm leaves reduced gas production when associated with *A. pungens* ($p<0.0001$), whereas this effect was less significant with *A. gombiformis*. This effect was more pronounced at earlier times of incubations. The *in vitro* organic matter digestibility decreased linearly with the increasing inclusion of date palm leaves in the mixtures. It is concluded that date palm leaves may be a suitable feed supplement for small ruminants browsing *A. pungens* and *A. gombiformis* in arid regions. The present results also showed that the inclusion level should be lower than 20% and for a short period in order to minimise tannins effect. The description of the fermentation profile of *in vitro* gas production showed that Sandoval model was poor and not appropriate for the characteristic varieties of arid and semi-arid areas in Algeria and the multiple regression models revealed a good linear regression for both mixtures.

Key words: Date palm leaves, forage, semi-arid zone, associative effects, rumen, *in vitro* fermentation.

INTRODUCTION

The problem of forage availability and quality of animal feed are aggravated in arid, semi-arid and tropical regions and because of the scarcity and irregular rainfall that limit the growth of herbaceous species and biomass

*Corresponding author. Email: deffairi.reu@Yahoo.fr.

yield in rangelands. Thus, breeding in these regions have to survive the shortage of insufficient resources for most parts of the year (Robles et al., 2008; Boufennara et al., 2012). 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 livestocks (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. This pathway provides to the rumen microorganisms the nutritive elements necessary for their growth, thus ensuring the favorable conditions for cellulolysis in the rumen (Moujahed et al., 2000).

In Algeria, the rangelands represent two-thirds of the total land widespread, mainly in the arid regions. Some species may be crucial for grazing ruminants where these plants contain anti-nutritional secondary compounds (phenolics and tannins) with potential side effects such as inhibition of rumen microbial fermentation, as well as decreased feed digestibility and animal performance (Min et al., 2003; Waghorn and McNabb, 2003; Mueller-Harvey, 2006).

In oasis areas, local farmers often offer date-palm leaves as a supplement for ruminants in spite of their high tannin content (Arhab et al., 2006). The anti-nutritive effect of dry leaves of the date palm has been studied in animal models of ruminants and mono-gastric and it seems that both reduced digestibility and toxicity may limit the potential of this plant as a feed supplement. The nutritional value of date palm crop residues has been quite extensively studied due to their high availability in the countries where date production is important (Alananbeh et al., 2015). Both the energy and protein values of these by-products are low as compared to that of cereal straw (Arbouche et al., 2008). However, no study has been conducted on the associative effect of palm leaves with other forage on in vitro fermentation.

The influence of different feed ingredients on rumen microbial activity can be variable and contradictory. For instance, increasing the level of cereal grain supplementation reduced ruminal fermentation of fiber (Mould et al., 1983a), whereas supplementation of alfalfa hay with corn stalks resulted in positive associative effects on N utilization that caused reduced intake of corn stalk (Wang et al., 2008). Rations formulated for ruminants are generally a mixture of individual feeds, and its net (NE) or metabolizable (ME) energy value is generally calculated by adding up the energy values of the individual feeds in it, on the hypothesis that the NE or ME value of individual feeds will be unchanged when they are fed in combination with other feeds. This assumption may not be true, as some published reports indicated that a level of association exists among feeds in rations (Dixon and

Stockdale, 1999; Franci et al., 1997; Haddad, 2000; Hong et al., 2001, 2002; Mould et al., 1983a, b; Wang et al., 2008). However, while associative effects are often discussed in ruminant nutrition, at least theoretically, they are seldom taken into account in feed formulation. While the meaning of an associative effect is clear (the sum of the parts being less, or more, than the combination of the parts), reasons for an associative effect are not clear. On the other hand, a true associative effect does not relate to correction of a known nutrient deficiency, such as fermentable N, but to unknown (or obscure) nutrient interactions.

In 1979, Menke et al. proposed an estimation of the energy value of feedstuffs from their in vitro gas production associated with chemical parameters. The gas test (HFT or Hohenheimer Futterwert Test) method is based on the assumption that the accumulated 24 h gas production by a substrate, incubated in a syringe with rumen liquor and a nutritive solution, is proportional to the amount of digestible carbohydrates, and thus highly correlated to the energy value of feedstuffs or to the in vivo organic matter digestibility (OMD).

The objectives of this study were to (i) investigate the associative effects on fermentation abilities of ruminal microbiota of sheep and gas production of plant leaves of the date palm and characteristics of two perennial plants in arid zones: Astragalus gombiformis and Aristida pungens (Stipagrostis pungens) mixed with different combinations and (ii) establish an interaction between the main components of different substrates.

MATERIALS AND METHODS

Forage material

Forages were collected from El Oued, located in South-East area of Algeria. The climate of this region is arid with a mean annual rainfall of 75 mm, and average temperature of 1°C in January and 43°C in July.

The studied forages consisted of two native species of North Africa: A. gombiformis (Foulet el Ibel), rich in protein (Arhab, 2006), A. pungens (Stipagrostis pungens) (drinn) rich in fibers (Arhab, 2006) and a by-product of the date palm: the leaves, rich in tannins (Arhab, 2006). These plants were harvested at a mature stage while date palm leaves were harvested at senescence. About six to ten specimens of each plant species were sampled to obtain a representative sample of the plant biomass.

The aerial part of the plants, leaves, thin branches (young stems) and some flowers (when existing) were clipped with scissors and taken immediately to the laboratory where the samples from the different specimens were pooled and dried in an circulating air oven at 60°C for 48 h. The samples were then coarsely ground in a laboratory using a chopper and were then ground again and passed through a 1 mm screen; the latter are mixed with the leaves of date palm at different percentages (0, 10, 20, 30, 40, 50 and 100%).

Animal material

The experiment was conducted on three Texel (origin of these

sheep Texel island) (breed of sheep), aged 12 months and an average live weight of 82 kg, deemed healthy by veterinary control, castrated and fitted with ruminal fistula. The animals were housed in individual pens. These subjects received a daily ration of 1200 g of oat vetch hay in two equal meals (8:00 am and 4:00 pm) with free access to water. Two weeks adaptation period were planned to allow a good adaptation of ruminal microbiota.

Analysis methods

Chemical analysis

The determination of the dry matter (DM) and organic matter of samples was conducted following the methods of AOAC (1999, 942.05 method ID). The analysis of the compounds of the plant cell wall was performed as described by Van Soest et al. (1991). The crude protein content of plants on one hand and content of crude protein associated with the fraction of neutral detergent fiber (NDF neutral detergent fiber) on the other were determined by the Kjeldahl method.

All determinations were conducted in triplicate and expressed as a percentage of DM. Phenolics were determined by addition of two or three drops of ferric chloride ($FeCl_3$) in 1 ml of the methanol extract that was diluted in 50% of distilled water. The change in color indicated the presence of phenolic compounds as follows: no change indicates absence of phenolic compounds, dark blue indicates presence of phenols or hydrolysable tannins, and dark green indicates presence of condensed tannins. The method followed was Folin-Ciocalteu as described in Julkunen-Tiitto (1985).

Quantitative analysis of phenolic compounds

Total phenols (TP) and total condensed tannins (TCT) was dosed separately.

Total Phenols (TP): The phenolic compounds analysis was carried out in three replicates. The dried plant material (200 mg) was extracted with acetone (10 ml, 70% v/v), then the solution was subjected, at ambient temperature for 20 min, to ultrasonic treatment. The content was centrifuged (4°C, 10 min and 3000 g) and stored in ice for analysis, then the centrifugate was treated as described above. The total phenols were estimated using Folin Ciocalteau reaction (Makkar et al., 1993). Tannic acid was used to perform calibration curve. TP was quantified as tannic acid equivalents and expressed as tannic acid eqg/kg DM.

Tanins: For the total condensed tannins (TCT), 0.5 ml of extract was treated with n-Butanol HCl (3 ml, 95%) in the presence of ferric ammonium sulfate (0.1 ml). The reactants were heated for 1 h in a boiling water bath. Absorbance was read at 550 nm. TCT were expressed as leucocyanidin using the equation:

TCT = Ax78.26x (dilution factor)/(weight of sample on DM)

where: A is the absorbance at 550 nm assuming that E1% efficiency, 1 cm, 550 nm is 460 leucocyanidin (Porter et al., 1986). Total tannins (TT) were determined as the difference in total phenolic compounds (measured by Folin-Ciocalteau reagent) before and after treatment with insoluble polyvinylpyrrolidone (Makkar et al., 1993).

In vitro study

The in vitro fermentation inoculum was obtained from the filtered rumen juice taken from three sheep. This juice was mixed to obtain a homogeneous inoculum. Ruminal fluid was collected 1 h before the morning meal, placed in a container preheated to 39°C and saturated with CO_2. This container was sealed immediately and transported to the laboratory for further analysis within 2 h of collection (Menke et al., 1979).

Sheep rumen fluid was mixed well, then filtered through four layers of muslin and bubbled with CO_2 at 39°C. All the handling was carried out under a constant stream of CO_2. The activity of ruminal microbiota requires an anaerobic and the solution was bubbled with a continuous flow of CO_2, resulting in the reduction of artificial saliva indicated by the color change from pink to transparent.

In vitro fermentation of forages by rumen microbiota

The technique followed was that of Theodorou et al. (1994). In vitro gas production technique is a simulation of food degradation by the rumen microflora. At the end of fermentation, the gas is measured using a pressure transducer (Delta Ohm DTP704-2BGI, Herter Instruments SL). It is a simple and inexpensive technique, based on the measurement of gas production.

In vitro gas production

The method of Theodorou et al. (1994) was used for gas production. Four hundred milligrams of each feed were weighed into bottles of 125 ml serum and incubated in a water bath at 39°C with 15 ml of ruminal fluid and 25 ml of artificial saliva (Van Soest, 1994). Monitoring fermentation kinetics was performed by measuring the gas pressure produced at different incubation hours: 3, 6, 9, 24, 48 and 72 h.

Statistical analysis

The evolution of gas production was followed according to the incubation time and the percentage of A. pungens or A. gombiformis in nutrient mixtures. A factorial ANOVA (the factorial ANOVA is based on design S <A*B> treating the effects of two crossed factors. The groups are assumed to be independent in each of the conditions defined by the intersection of two factors. The sources of variation to take into account are the factors A (time) and B (percentage) and optionally AB (time*percentage) interaction) was performed to find out the effects of the incubation time, the percentage of added plants and their mutual interaction on gas production. A fermentation profile model from the literature, based on Levenberg-Marquardt algorithm (Levenberg, 1944; Marquardt, 1963) which is an improvement of the Gauss-Newton method for the resolution of nonlinear least-squares regression problems, was tested. Then, a multiple regression was performed to determine the equation giving the best estimate of gas production based on the independent variables and their interaction. Statistical analyses were conducted with Statistica 10, Statsoft Inc, Tulsa, USA.

RESULTS AND DISCUSSION

The chemical composition of forages

The chemical composition of the substrates is shown in Table 1. There was a change in all nutrient elements of forages. The highest fiber content was recorded in A. pungens (Stipagrostis pungens) (509.4 g/kg DM),

Table 1. Chemical composition of simple fodder (in g/kg DM).

Forages	DM	CP	NDF	ADF	ADL	TP	TT	TCT	PPC
A. gombiformis	551.7	125.0	614.9	445.2	78.1	34.0	21.3	4.0	ND
A. pungens	797.3	53.6	794.7	509.4	84.4	2.4	1.6	3	ND
Date palm leaves	896.3	59.5	586.1	422.1	97.1	61.8	49.1	36.2	55.5

DM, Dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, lignin determined by solubilisation of cellulose with sulphuric acid; TP, total phenols; TT, total tannins; TCT, total condensed tannins; PPC, protein precipitating capacity.

followed by *A. gombiformis* (445.2 g/kg DM) and the leaves of dry palms (422.1 g/kg DM). The highest crude protein content was noted in *A. gombiformis* (125 g/kg DM) followed by palm leaves (45.9 g/kg DM). Regarding phenolic compounds (total phenols, TT and TCT) were 61.8, 49.1 and 36.2 g/kg DM for dry palm leaves, by *Astragalus* and *A. pungens*, respectively. The change in the mineral and organic elements concentration for the studied plants was strongly associated with the type of soil, climate, stage of maturity and harvest season genotypic characteristics, and the factors affecting the nutritional properties of forages (Arhab, 2007; Bahman et al., 1997; Pascual et al., 2000; Genin et al., 2004; Ramirez et al., 2004; Ammar et al., 2004).

According to the level of crude protein, *A. gombiformis* had high nitrogen level; in fact it was richer than the green forage such as *Setaria sphacelata* in which nitrogen content was between 47.4 and 69.2 g/kg of DM (Rakotozandriny, 1993). However, *A. gombiformis* is considered as the most digestible plant with a high content of crude protein (125 g/kg DM), which shows that the forage resource has an interesting nutritional potential for ruminants (Boufennara, 2012). On the other hand, Pascual et al. (2000) and Genin et al. (2004) showed that the high level of protein in *A. gombiformis* indicates its possible use as a protein supplement for ruminants.

The high content of the cell wall could be preserved because of the climate in the arid zone. In general, high temperatures and low rainfall tend to increase cell wall polysaccharides and decrease the soluble carbohydrates (Pascual et *al.*, 2000).

The concentration of phenolic compounds varies considerably among plant species, the highest levels were observed in the dry leaves of the date palm. This could be due to the fact that the radial diffusion method, based on the measurement of the potential biological activity of tannins in food, will depend on the bonding force of the tannins and their mode of binding to the protein (Frazier et al., 2003), while chemical methods, based on the chemical properties of tannins, indicate the chemical nature of tannins (Silanikove et al., 1996).

Chemical composition of mixtures

For mixtures, it was noticed that the addition of palm

leaves increased the DM level especially the level of tannins (TT and TCT) of mixtures and reduced the levels of fiber and crude protein in proportion to percentages (Tables 2 and 3). This can be explained by the ability of tannins to form complexes with proteins and therefore the fall of the levels of proteins (Dalzell and Kerven, 1998).

Furthermore, it was found that for less percentage of mixtures (10-20%), TCT and TT levels were low, 7.22-10.44 and 24.08-26 86 g/kg DM, respectively, while the level of total nitrogen contents was higher (118.5 and 111.9 g/kg DM) in the palm leaves mixture and *A. gombiformis* as compared to those obtained in the leaves of date palm only.

For the mixture of date palm leaves and *A. pungens*, it was noted that at the same percentage of incorporation, the level of the nitrogen material was low as compared to the first mixture (54.2-54.8 g/kg DM) while the tannins levels were similar for both mixtures. According to a research carried out by Paterson et al. (1996), forages which have MAT contents below 70 mg/g DM require nitrogen supplementation to improve their ingestion by ruminants.

Gas production

The gas production of mixture of the date palm leaves with *A. pungens* (*S. pungens*) or *A. gombiformis* at different percentages of fermentation for individual substrates and mixtures are presented in Figures 1 and 2. The results of factorial ANOVA showed that adding palm leaves decreased gas production when they were associated with *A. pungens*, with significant effects ($P < 0.0001$), whereas, this effect was significant but less pronounced with *A. gombiformis*. For the mixture of the leaves of date palms with palm *A. pungens*, a highly significant effect of time ($F=1341.6$, $p<0.0001$), percentage ($F=23.64$, $p<0.0001$) and interaction of time with percentage ($F=7.28$, $p<0.0001$) was found.
For the mixture of the leaves of date palms with *A. gombiformis*, there was a highly significant effect of time ($F=323.5$, $p<0.0001$), percentage ($F=10.84$, $p<0.0001$) while no effect of interaction of time with percentage ($F=0.78$, $p=0.785>0.05$) was found.

Indeed, there is an increase in the amount of gas generated as a function of time. The cumulative volume

Table 2. Chemical composition of the mixture (*Aristida* and palm leaves).

	A. pungens	Feuilles	0% Palm	10%	20%	30%	40%	50%	100%
MS g/kg	7973	8963	797,3	807,2	817,1	827	836,9	846,8	896,3
MM g/kg	119,6	109,5	119,6	118,6	117,6	116,6	115,6	114,6	109,5
Azote	8,56	9,5	8,56	8,65	8,75	8,84	8,94	9,03	9,5
MAT (Nx6,25)	53,6	59,5	53,6	54,2	54,8	55,4	56	56,6	59,5
N-NDF	3,75	5,3	3,75	3,91	4,06	4,22	4,37	4,53	5,3
Azote libre	4,81	4,2	4,81	4,75	4,69	4,63	4,57	4,51	4,2
NDF	794,7	568,1	794,7	772	749,4	726,7	704,1	681,4	568,1
ADF	509,4	422,1	509,4	500,7	491,9	483,2	474,5	465,8	422,1
Hémicelluloses	285,3	164,9	285,3	273,3	261,2	249,2	237,1	225,1	164,9
ADL	84,4	97,1	84,4	85,7	86,9	88,2	89,5	90,8	97,1
Cellulose	425	324	425	414,9	404,8	394,7	384,6	374,5	324
TP	2,4	61,8	2,4	8,34	14,28	20,22	26,16	32,1	61,8
TT	1,6	49,1	1,6	6,35	11,1	15,85	20,6	25,35	49,1
TCT	3	36,2	3	6,32	9,64	12,96	16,28	19,6	36,2
PPC	ND	55,45	ND						55,45

Table 3. The chemical composition of mixture of *A. gombiformis* and palm leaves.

	A. gombiformis	Feuilles	0% Palm	10%	20%	30%	40%	50%	100%
MS g/kg	551,7	896,3	551,7	586,2	620,6	655,1	689,5	724	896,3
MM g/kg DM	231,7	109,5	231,7	219,5	207,3	195	182,8	170,6	109,5
Azote	20	9,5	20	18,95	17,9	16,85	15,8	14,75	9,5
MAT (Nx6,25)	125	59,5	125	118,5	111,9	105,4	98,8	92,3	59,5
N-NDF	6,4	5,3	6,4	6,29	6,18	6,07	5,96	5,85	5,3
Azote libre	13,6	4,2	13,6	12,66	11,72	10,78	9,84	8,9	4,2
NDF	614,9	568,1	614,9	610,2	605,5	600,9	596,2	591,5	568,1
ADF	445,2	422,1	445,2	442,9	440,6	438,3	436	433,7	422,1
Hémicelluloses	122,7	164,9	122,7	126,9	131,1	135,4	139,6	143,8	164,9
ADL	78,1	97,1	78,1	80	81,9	83,8	85,7	87,6	97,1
Cellulose	356,4	324	356,4	353,2	349,9	346,7	343,4	340,2	324
TP	34	61,8	34	36,78	39,56	42,34	45,12	47,9	61,8
TT	21,3	49,1	21,3	24,08	26,86	29,64	32,42	35,2	49,1
TCT	4	36,2	4	7,22	10,44	13,66	16,88	20,1	36,2
PPC	ND	55,45	ND						55,45

of gas increased with the increase of incubation time (Figures 1 and 2). Significant differences in gas production were found between the substrates for all the incubation times. Gas production decreased in the presence of dry palms; this could be explained by the fact that they contained substances (tannins) that affect the hydrolytic way (enzymes) of *A. pungens* (mainly cellulose enzymes) (Sweeney et al., 2001). The best gas production can be obtained at high time but for low palm percentages (10 and 20%); according to Robinson et al. (2009). Gas production was much more pronounced in the early hours of incubation with mixture levels of 15 to 25%; this effectively proves that the incorporation of dry palms inhibited *A. pungens* degradation by ruminal microbiota. This can be explained by the chemical composition of dry palms in tannins. Indeed, effects of this are well established on fermentation activity. According to McSweeney et al. (2001), tannins can directly influence the ruminal microbiota and enzyme activity; in fact, condensed tannins exert an inhibitory action on growth of rumen microorganisms. Furthermore, Makkar and Becker (2009) suggested that TT have an influence on the reduction of methane production.

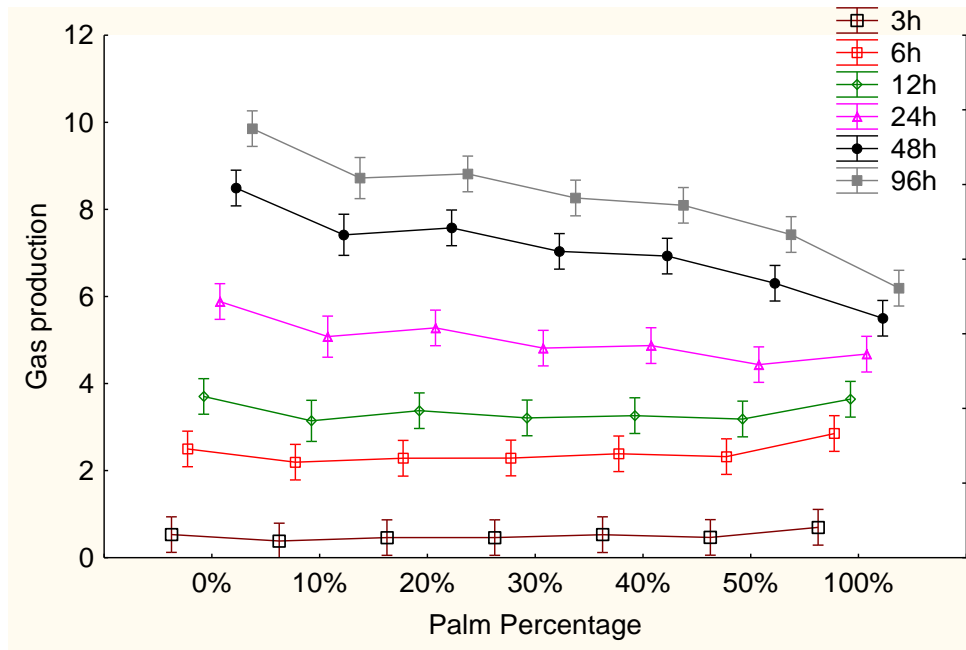Unlike the association of palms-drinn, palms did not

Figure 1. Gas production based on percentage of date palm leaves and *A. pungens* (*S. pungens*) at different times.
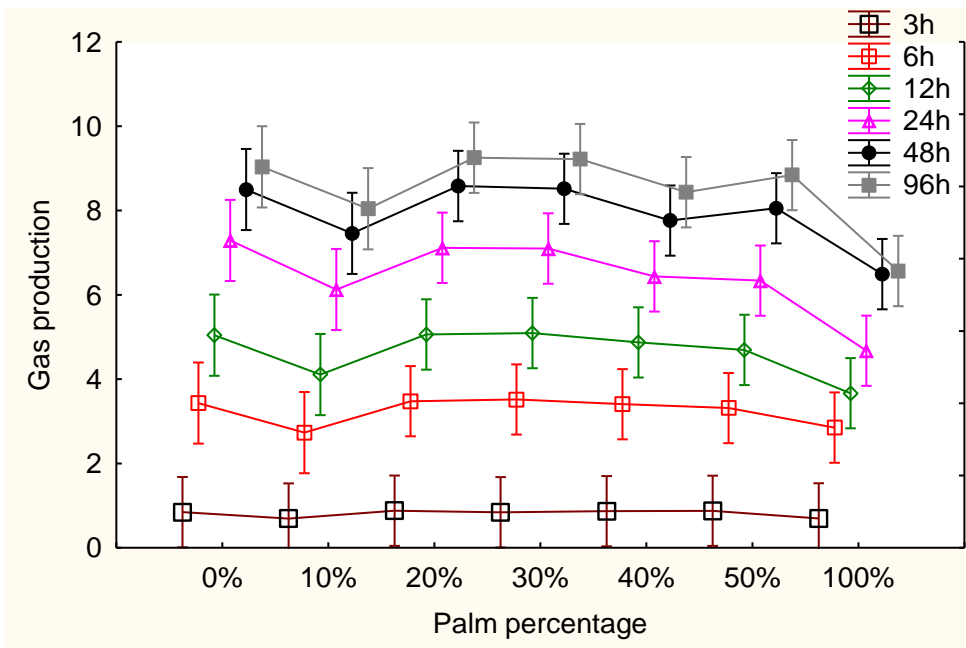

Figure 2. Gas production based on percentage of date palm and *A. gombiformis* at different times.

affect the fermentation of *A. gombiformis*. This may be explained by the fact that dry palms, rich in tannins, do not affect the proteolytic enzymes (specificity of action and structure), or that the chemical composition of *A. gombiformis* limits the effects of palm leaves on the fermentation parameters. *A. gombiformis* is rich in

Table 4. Results of the Levenberg-Marquardt estimation method with convergence criteria at 10^{-9}.

Mixture (% Drinn)	a	b	R^2	Mixture (% *A. gombiformis*)	a	b	R^2
0	-3.765	8.551	0.961	0	-4.154	9.234	0.961
50	-2.896	7.958	0.937	50	-7.490	13.924	0.973
60	-3.702	9.162	0.945	60	-7.066	13.402	0.973
70	-3.276	8.764	0.936	70	-7.054	13.864	0.970
80	-3.512	9.351	0.936	80	-8.031	14.941	0.973
90	-2.432	7.935	0.905	90	-4.953	10.984	0.948
100	-4.499	10.932	0.945	100	-7.418	14.236	0.965

a and b are the two coefficients of (Gas prod)=$a+b(1-e^{-kt})$, where k is the hourly rate of the gas production (%.t), t the time (h), R^2 is the coefficient of determination.

nitrogen matter, it generates the production of biomass. Indeed, the degradation of crude protein of *A. gombiformis* generates biomass production. Liu et al. (2002) demonstrated in an *in vitro* system, that an amount of nitrogen is sufficient to sustain microbial growth. In addition, it has been shown that the nitrogen can make only a small contribution to gas production. According to McSweeney (2001), the nitrogen digestibility in ruminants reported favorable responses when providing as supplement. Wang et al. (2008) had shown that supplementation with nitrogen increase digestibility of forage.

Our results are consistent with those reported by Aregheore et al. (2000), Long et al. (1999) and Khazaal et al. (1993). These authors report that the contribution of the total nitrogenous matter (MTA) on gas production is not a significant influencing factor. This suggests an intensification and stimulation of rumen fermentation activity of the microbiota when the latter is in the presence of a food rich in nitrogen and energy (Tendonkeng, 2004; Getachew et al., 2000 and Florence et al., 1999).

Otherwise, supplementation with greater amounts of energy-rich feeds with a source of protein could reduce the time taken to finish cattle for market and increase profitability. Numerous reports in the literature indicate substantial increases in live weight (LW) gain by supplementing cattle consuming low digestibility forages with energy and protein supplements (Hennessy and Murrison, 1982; Lee et al., 1987; Hennessy et al., 1995).

A positive effect on associative gas production has been reported when the leaves of forage trees were mixed with concentrate diets (Sandoval-Castro et al., 2002) and when the straw was mixed with tree leaves (Liu et al., 2002).

Modeling of gas production

The model of Sandoval-Castro (2000) was used for the description of the fermentation profile of the *in vitro* gas production. Therefore, the authors tried to stimulate the development of gas production according to the equation: (Gas Prod) = $a + b(1-e^{-kt})$ based on the percentage of *A. pungens* or *A. gombiformis*. The estimation method is that of Levenberg-Marquardt where a and b were two coefficients to be determined, k the hourly rate of gas production (%.t) and t the time. The results are summarized in Table 4. The coefficients determination R^2 were always >0.90. Negative values of "a" (Orskov and Ryle, 1990) were noted. According to several authors, a negative value of "a" is the result of the existence of a latent phase during which microorganisms attach and colonize food particles before any damage is detectable (Ahmed and El-Hag, 2004). This could also indicate the difficulty of ruminal microbiota to degrade these substrates and the need for an adjustment period so that it begins this degradation.

Despite the values of negative "a" and high coefficients of determination R^2>0.90, the convergence remained mediocre. Accordingly, this theoretical model remains poor, inflexible and not suitable for varieties of arid and semi arid areas. This mathematical model does not apply to plants of Saharan zones. That is why the authors proposed the model of multiple regression (Figures 3 and 4) with a relationship type Gas Production = $a + bt + cP + dPt$ for the two mixtures where a, b, c and d are constant coefficients to be determined, t the time (h) and P the percentage of *A. pungens* or *A. gombiformis*. The analysis showed a good regression with multiple r = 0.896, with highly significant effects of t and interaction Pt ($p<0.0001$), but with non sensible effect of the percentage ($p=0.365$). Gas Production = $2.332 + 0.045t − 0.395P + 0.0453Pt$ for mixtures of leaves palm/*A. pungens*. For the palm mixture with *A. gombiformis*, a good regression with multiple r = 0.799, with significance of t ($p<0.001$), but with no sensible effect of P or interaction Pt ($p=0.41$ and $p=0.10$ respectively) was found. The equation was Gas Production = $2.723 + 0.0548t + 0.518P + 0.0233Pt$. So, the regression model can be considered as available
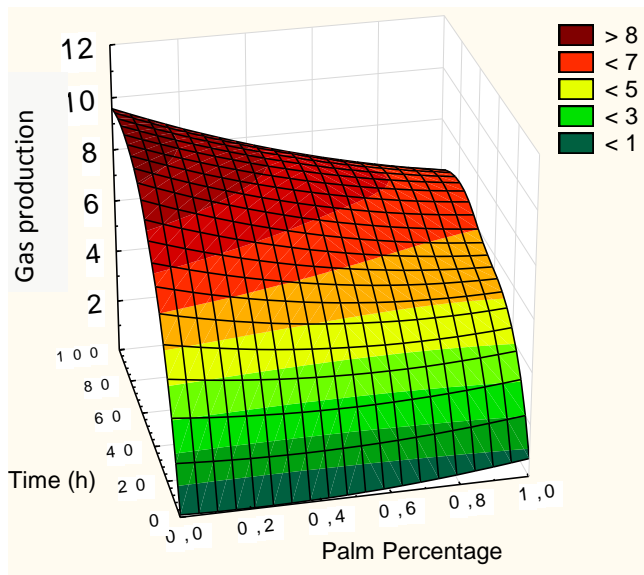
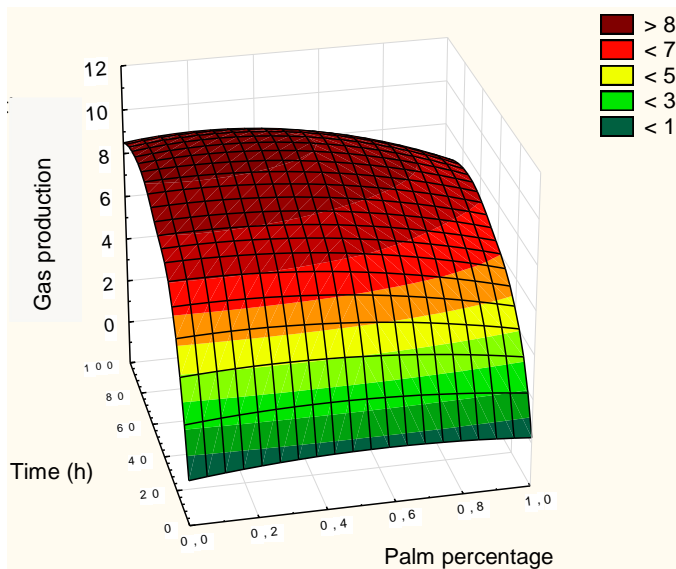**Figure 3.** Rate of gas production of dry palm leaves and *A. pungens* mixtures.

Figure 4. Rate of gas production of dry palm leaves and *A. gombiformis* mixtures.

for both mixtures.

Conclusion

This study has shown that associative effects on *in vitro* gas production are consistently higher when the leaves of the date palm were incubated with *A. gombiformis* than

with *A. pungens* (*S. pungens*). In addition, palm leaves can be a food supplement suitable for small ruminants browsing *A. gombiformis* or *A. pungens* (*S. pungens*) in arid regions. The present study results also demonstrated that the percentages of the incorporated palm leaves had to be less than 20% and for short periods in order to minimize the effect of tannins. The description of the fermentation profile of *in vitro* gas production revealed that Sandoval (2000) model is poor and therefore not appropriate for the characteristics of varieties of arid and semi-arid areas in Algeria, whereas the model of multiple regression has revealed a good linear regression for the two types of mixtures.

Conflict of Interests

The authors have not declared any conflict of interests.

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