



## Effect of inoculum source (Sheep or Goat) on in vitro gas production and rumen fermentation parameters of some fodder shrubs in northern Tunisia

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### ABSTRACT

In order to ensure nutritional characterization of some fodder shrubs and test the effect of inoculum source (sheep and goats) on their digestibility. Four shrubs "Acacia cyanophylla, Atriplex halimus, Opuntia ficus-indica. Var.inermis(cactus) and Medicago arborea" were tested in vitro by incubating them firstly into the rumen fluid of sheep then into goat's inoculums. The evaluation included chemical composition, total gas production (CO<sub>2</sub> and CH<sub>4</sub>), the prediction of organic matter digestibility (OMD), metabolizable energy (ME), the concentration of total volatile fatty acids (SCFA) and metering ammonia nitrogen (NH<sub>3</sub>-N). Ruminant fermentation of Opuntia ficus-indica and Medicago arborea have identified more total gas amounts than Atriplex halimus and Acacia cyanophylla. Digestibility of organic matter, metabolizable energy and the concentration of total volatile fatty acids were higher in the case of Opuntia ficus-indica compared to other shrubs. The lowest values were recorded in the Atriplex halimus. The concentration of ammonia nitrogen (NH<sub>3</sub>-N), it turned out that Opuntia ficus-indica is the food with less protein concentration (140 and 270.66 mmol/syringe) respectively for sheep and goats. In against part, the Medicago arborea is presented as a good protein source recording of ammonia nitrogen concentration (214.67 mmol/syringe) into the sheep's rumen and (494.67 mmol/syringe) into the goats inoculum. The source of the inoculum showed a highly significant effect (p<0.05) of the production of gas and the concentration of ammonia nitrogen. Goats presented the highest gas quantities and concentration of NH<sub>3</sub>-N for all tested shrubs. The difference between the rumen fluid on DOM, ME and VFA was not significant (p> 0.05).

**Key words:** Digestibility, Fodder shrubs, Goats, Ruminant fluid, Ruminant fermentation, Sheep.

### Academic discipline sub-disciplines

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## INTRODUCTION

Ruminants are an important food source for humans. Indeed, they ingest some plant species (forage), the man can not benefit directly. This means that important has always been the object of scientific research in order to understand how their organization to better meet their needs. Spontaneous vegetation, especially woody, are an important contribution to cover the needs of ruminants that live there. To make optimal use of the nutritional potential of this natural vegetation, it is necessary to know its nutritional value [Boubaker et al., 2004].

Nefzaoui and Chermiti [1991] indicate that planting fodder shrubs are undeniably an element of stability in the supply of small ruminants through inter annual carry forage accumulated over 2-3 years and used in case of drought. They can be considered, in many circumstances, as the main food for animals in some traditional farming systems in mountainous areas and arid areas. This idea can be confirmed by Kibria et al. [1994], which state that in some extensive sterile areas and mountain systems, forage obtained from shrubs can happen to represent the most important protein intake and energy for ruminants and especially for goats. In addition, plantations shrubs are an investment in the medium and long term and renewable resource, while the price of concentrates and the rate resulting from economic situations.

These arguments highlight the importance of fodder shrubs that provide a standing biomass regularly throughout the year. This biomass is less subject to seasonal and annual variations of herbs [Nefzaoui and Chermiti, 1991].

The contribution of forage shrubs in meeting the food needs of animals differs from a shrub species to another. For example, studies conducted by El Euch [2000] showed the important role played by the Tunisian *Acacia cyanophylla* plantations in livestock feed helping to cover the overall energy needs of about 145.000 heads of sheep or goats' year taking into account the total production of these plantations is estimated at 43 million UF / year and energy needs.

## Materials and methods

### Shrub species

Four tree species: *Atriplex halimus*, *Acacia cyanophylla*, *Medicago arborea* and *Opuntia ficus indica* were used. Samples the month of November has improved from a course belonging to the farm Sawaf- Zaghouan area north of Tunisia. The samples were dried at 40 ° C for 48 h did not affect the amount of tannins and their biological properties [Makkar and Singh, 1991] and ground to a 2 mm sieve.

### Source and collection of the inoculum

The removal of the inoculum of the two animal species (sheep and goats) studied was performed by the same technique. The rumen fluid taken orally by pressure force on fasting adult animals by administering directly in the rumen a plastic tube connected to a syringe made of polypropylene. The rumen content is then homogenized and then filtered through four layers of surgical gauze to remove the solid phase.

### Chemical composition and nutritional value

Samples of different plant species were analyzed for their dry matter (DM), organic matter (OM), mineral mixture (MM), total nitrogenous matter (CP) and lipids (FAT) according to [AOAC, 1990]. The total fiber (NDF) were obtained after dissolution under the action of a neutral detergent sodium content of Acid Detergent Fiber (ADF) was measured in the presence of cetyl tri methyl ammonium bromide and lignin (ADL) was determined on the ADF residue subjected to the action of sulfuric acid solution at 72% [Van Soest et al., 1991]. The relationship between the levels of total plant walls (% DM) based on the total crude fiber (CF is determined by reference to the table of [INRA, 2007]. The prediction of food values (UFL / kg DM, PDIE g / kg DM and PDIN g / kg DM) according to the formulas of [Andrieu et Weiss, 1981].

### In vitro gas production

To study the characteristics of *in vitro* gas production [Menke and Steingass, 1988], rumen fluid was obtained from four rumen sheep and goats fed oat hay (70%) and concentrates (30%). The inoculum was mixed with a buffer solution in a ratio of 1:2 (v/v). About 300 mg of dry samples were introduced into calibrated syringes, and 30 ml of the buffered inoculum were sucked through a silicone tube attached to the needle top of each syringe. Then the gas bubbles were removed and the silicone tube was clamped, the position of the lubricated piston was recorded, and the syringes were placed in the oven at 39 °C. Gas production was recorded at 3, 6, 12, 24, 36, 48, 72 and 96 h. The ME of seeds and SCFA produced in syringe were calculated as follows:

$$\text{OM digestibility (g/100gMO)} = 14.88 + 0.889 \text{ Gp} + 0.45 \text{ CP} + 0.0651 \text{ C}$$

$$\text{ME (MJ / Kg DM)} = 2.20 + 0.136 \text{ Gp} + 0.057 \text{ CP} \text{ [Menke and Steingass, 1988]}$$

$$\text{SCFA (mmol/syringe)} = 0.0239 \cdot \text{Gas} - 0.0601 \text{ [Getachew et al., 2000]}$$

Where ME is the metabolizable energy; SCFA: short chain fatty acids; CP, crude protein in %

And Gp, the net gas production in ml from 200 mg dry sample after 24 h of incubation.

The concentration of ammonia nitrogen was analyzed by the method of Conway (1962) as follows:  $\text{N-NH}_3 \text{ Mg / ml of rumen juice} = (\text{V E HCL} - \text{V HCLC}) * \text{N (HCl)} * 14$ .



## Statistical analysis

The results of the effects of fodder shrubs (*Atriplex halimus*, *Acacia cyanophylla*, *Medicago arborea* and *Opuntia ficus indica*) and those sources of inoculum (sheep and goat) on the measured parameters were subjected to analysis of variance according to the procedure GLM of SAS [1989] and compared by the multiple range test of Duncan [1955]. The characteristic parameters of the gas production kinetics were predicted according to the non-linear regression model of Orscov and McDonald [1979]:  $Y = a + b * (1 - e^{-ct})$ .

## Results and discussion

### Chemical composition and nutritional value

*Atriplex halimus* has a high value in CP (20.4% DM) compared to other shrubs ( $p < 0.05$ ), whereas *Opuntia ficus indica* shows the lowest value. The results of these joined [Nefzaoui and Chermiti, 1991] who showed that these shrubs present values of CP comparable to raw materials such as faba bean and lupine. The content of fatty acids was statistically comparable for the four shrubs ( $p > 0.05$ ). These values are similar to the FAT content of the cereal grains. Mineral matter content was significantly higher ( $p < 0.05$ ) for *Atriplex halimus* and *Opuntia ficus indica* and conversely the organic matter content is lower for these two shrubs with significant difference between the shrubs studied ( $p < 0.05$ ). This result is similar to what has been suggested by Ben Salem et al. [2004]. Regarding the fiber content, laboratory analysis reveals that the *Medicago arborea* has the highest levels in CF (32.5), NDF (50.7) and ADF (33.5) compared to other shrubs ( $p < 0.05$ ), while *Opuntia ficus indica* displays the lowest values in different type of fiber ( $p < 0.05$ ). This difference reflects the difference in fiber digestibility of these shrubs. Indeed, *Opuntia ficus indica* The energy value of forage shrubs studied ranges from 0.78 to 0.94 UFL / kg DM. This is consistent with the results of Nefzaoui and Chermiti, [1991]. *Acacia cyanophylla* presents the highest value which is comparable to that of feed concentrates. This can be explained by its nutritional value (CP 14 to 15% of CF and 25 to 30%) and a relatively high palatability especially after wilting, *Acacia cyanophylla* is undoubtedly a promising species for the semi-arid Tunisia. However, its operating time (September to the end of winter) does not coincide with periods when its chemical composition and palatability are optimal (spring, summer). *Medicago arborea* has a lower energy value compared to other shrubs is what can be explained by its fiber content is higher. *Opuntia ficus indica* displays the lowest amounts in PDIN and PDIE it is related to its low CP.

### In vitro gas production

Table 1 shows the volume and the characteristic parameters of gas production from after incubation of fodder shrubs in both sheep and goats inoculated. According to statistical analyzes, it is apparent that the production of gas (total gas) in vitro, vary significantly ( $p < 0.001$ ) in each inoculum incubated according shrubs. This indicates that the variability of the fermented in the same inoculum volume of food influences the gas released therein. The difference noted for the total volume of gas produced is certainly associated with the different chemical composition of the substrates studied vary depending on the species and the botanical family.

After incubation for 96 hours, it appears that among fermented substrates, *Opuntia ficus-indica*. *Var.inermis cactus* generates the largest amount of gas (63.3 ml / 0.3 g Ms) and (70.33 ml / 0.3g MS) respectively inoculum in the sheep and goat. In both incubation media, it is always followed by the tree lucerne whose respective outputs are 57.6 ml / 300 mg DM and 62.33 ml / 0.3 g DM. Therefore, we can conclude that the spineless *Opuntia ficus-indica*. *Var.inermis cactus* and *Medicago arborea* are the most fermentable by rumen microflora shrubs. This leads to better classify as digestible by small ruminants.

For both animal species (sheep and goat), the values recorded in *Atriplex* and *Acacia* are distinguished by the amount of gas the lowest, they are respectively in the range of 37ml / 0.3 g DM and 41 ml / 0.3 g DM in sheep inoculum and 45 and 47.33 ml / 0.3 g DM in the goats.

The superiority of gas production spineless *Opuntia ficus-indica*. *Var.inermis cactus* compared to production of other shrubs can be explained by its high soluble sugars (carbohydrates cell content) rapidly fermentable.

The *Medicago* is also very fermentable as a product similar to *Opuntia ficus-indica*. *Var.inermis cactus* volume. This seems to be due to its classification as a leguminous plant that is on the one hand, rich in structural carbohydrates because of its high fiber content (% NDF) which are substantially free of lignin (ADF - CBT) known as very difficult to degrade substance. On the other hand, *Medicago* is rich in protein (16.2%) due to its ability to fix atmospheric nitrogen. This leads to a stimulation of fermentation activity of ruminal microbiota in the presence of a rich faith in nitrogen and energy food. This was proved by Guetachew et al. [2000].

Despite belonging to the same order (Fabales), and their chemical compositions similar viewpoint MO, MAT and structural carbohydrates, we note that the gas production of alfalfa tree is much higher than that recorded in the *acacia cyanophylla*. This can be explained by the richness of the latter species by tannins that are condensed and deteriorating food spoilage by microorganisms in the rumen. The content of the *acacia* in this anti-nutritional substance was announced by Reed et al. [1990]. Indeed, the digestibility of fodder and some wood tannins particularly bind to proteins and dietary fibers with which they form complexes resistant can not be degraded by the rumen microbiota.

The kinetic parameters of the in vitro fermentation of different substrates derived from the Orscov and McDonald model, are also mentioned, we see that the factor values (a) are significantly different in the same incubation medium, they are generally positive for most substrates shrubs. The only negative value (-0.487) was registered in *Atriplex* sheep in the inoculum, this observation is similar to that found by Ben Salem et al. [2004] was of the order of 2.6 ml / 200 mg MS.



According to Ahmed and El-Hag, [2004], the negative value of (a) is the result of a lag phase during which microorganisms attach and colonize the food particles before their eventual degradation.

This value shows that the volume of gas released from the fraction soluble. Its highest value is found in the *Opuntia ficus-indica*. *Var.inermis cactus*, it is 6.13 and 5.64 ml / 0.3 g DM respectively in the two inocula (sheep and goat). Although it did not produce a large volume of gas, acacia has a value of (a) significant (4.57 ml / 0.3mg MS in sheep and 4.54 ml / 0.3mg MS in goats). This can be explained by improper fermentation of the insoluble fraction.

The volume of gas produced from the insoluble but potentially fermentable fraction (b) is statistically different ( $p < 0.001$ ). The largest volume marks the *Opuntia ficus-indica*. *Var.inermis cactus* 56,23ml / 300mgMS in sheep and 67.97 ml / 0.3 g Ms in goats, intermediate values characterize the tree lucerne and *Atriplex*. The acacia is characterized by the lowest degradation of its insoluble fraction and produces only 34.7 and 42,3ml / 300mg MS respectively in sheep and goats. The observed relationship between forage shrubs studied in the fermentation of their parietal fractions difference is due to their different chemical composition. Indeed, the low gas production from the fraction (b) observed in acacia may be due to its high content of NDF fraction (46%). To this effect probably added action of tannins (antinutrients) that can reduce fiber degradation by complex formation with the lignocellulosic fraction, thus preventing the adhesion of microorganisms. They also inhibit the degradation involved in cellulases or cellulolytic bacteria directly by forming complexes with minerals required for growth [Hervas et al., 2003].

Speeds of fermentation insolubles studied substrates are quite different ( $p < 0.05$ ), the tree alfalfa expresses its ability in field by inducing higher speed:  $0,074h^{-1}$  in sheep and  $0,048 h^{-1}$  in goats. Values slightly below that are noted for the *Opuntia ficus-indica*. *Var.inermis cactus* in both inoculated with  $0,065h^{-1}$ . Fermentation, although the tree lucerne fast compared to other shrubs can be explained both by its insoluble fraction bit woody and easily accessible to ruminal microbiota. The fermentation rate is on the intake of food as it affects the speed of passage of feed particles in the rumen [Jouany et al., 1995]. These results are consistent with studies [Monjauze and Hou  rou, 1965] working on the *Opuntia ficus-indica*. *Var.inermis cactus* and found that the food is very palatable to sheep that can ingest up to 2.5 to 9 kg / day of snowshoeing.

### Ruminal fermentation parameters

Depending on the volume of gas produced in 24 hours of incubation, it was determined, the values of metabolizable energy (ME), the digestibility of the organic matter (OMD) and the production of total volatile fatty acids (AGVT). We note that all the estimated parameters vary significantly depending on each shrub studied. The values of the coefficient of determination  $R^2$  are in no case less than 0.8, it contains at least 80% of the variation of each parameter (MS, MO and AGVT d) is explained by the variability of the characteristics of fodder shrubs including the variability of their chemical compositions and their food value. The digestibility of the organic matter (OMD) is variable through the various plants tested (Table 2). The greatest digestibility coefficients were recorded for the *Opuntia ficus-indica*. *Var.inermis cactus* (63.39% and 60.14%) and the tree lucerne (63% and 61.8%). These results are higher in comparison with those found by Kamalak [2005] worked on a reference food such as oat-vetch hay and gave him a dMO 58.38%. Percentages of (OMD) characterized least acacia (50.25% and 52.9%) and *Atriplex* (50% and 47.6%). So substrates rich in tannins and anti-nutrients are the most affected, in fact several authors report a negative correlation between OMD and tannins [Frutos et al., 2002; Seresinhe and Iben, 2003; Kamalak, 2005].

Digestibility of OM seems positively correlated with the production of fatty acids Voltails and ME. This is explained by the placement of all sources of energy and protein in this fraction, which is a good digestibility of the latter leads to a better energy supply.

For both sources of inoculum The highest values of ME were recorded in the spineless *Opuntia ficus-indica*. *Var.inermis cactus* and *Medicago* with 2089.34 and 2208 Kcal / Kg MS for *Opuntia ficus-indica*. *Var.inermis cactus* and 2220 and 2076 Kcal / Kg for MS the *medicago*. These values were observed respectively in sheep and goats. This result can be explained primarily by the high energy value of *Opuntia ficus-indica*. *Var.inermis cactus* seen his wealth by easily fermentable soluble carbohydrates and making a significant amount of energy. Second, the richness of the tree lucerne MAT in addition to a high content of cell wall carbohydrates with a low lignin.

Acacia and *Atriplex* remain classified in second place with a slight superiority of the first plant. This classification may be caused by the presence of secondary substances and excessive concentration of salts in *Atriplex* (100 to 200 g NaCl per day can be consumed by sheep fed *Atriplex*), which may limit its nutritional value [Nefzaoui and Chermiti, 1989] adversely affecting rumen microbial activity and the concentration of acacia tannins that can inhibit the degradation of structural carbohydrates by focusing on the fiber forming non-degradable complexes.

Volatile fatty acids are the source of energy used by ruminants, they are the end products of rumen digestion of dietary carbohydrates which include various compounds derived from either the plant cell wall, such as cellulose, hemicellulose and pectins or the cell contents such as soluble sugars. Their concentration depends on the amount of energy provided by the food as well as the quality of the carbohydrate contained therein. These characteristics explain the superiority of the concentration AGVT observed in *Opuntia ficus-indica*. *Var.inermis cactus* and *medicago* which are ranging between 0.99 and 1.1 mmol / syringe concentrations. From what is mentioned in the table above, we note that the classification of the nutritional value of fodder shrubs is comparable to that derived from the analysis of the total gas production: *Opuntia ficus-indica*. *Var.inermis Cactus* tree are spineless and alfalfa most interesting in terms of energy as acacia and *atriplex*.

From Table 3, it is apparent that the ammonia nitrogen concentration varies significantly depending on the animal species which are collected rumen juice. For all fodder shrubs were recorded higher concentrations of  $NH_3-N$  in sheep than in



goats. Indeed, the production of ammonia nitrogen Medicago is a legume rich in protein can be seen, pass 494,6g / L in sheep 214,6g / L in goats. This result may be due to extensive use of ammonia by the ciliated protozoa that invade the rumen environment of goats. There will be a good spread of the types of microorganisms that will contribute substantially to the digestion of the cell walls of the fibers and the total gas production.

## Conclusion

The results led to the conclusion that the fodder shrubs northern Tunisian food can be a good alternative, especially for small ruminants. Indeed, with good nutritional value, digestibility significant and fairly high palatability. Medicago arborea can be without doubt a promising species for wet areas. The Acacia cyanophylla and spineless Opuntia ficus-indica. Var.inermis cactus can mutually compensate for their shortcomings and form a well-balanced diet thanks to the wealth of Opuntia ficus-indica. Var.inermis cactus water, carbohydrates and vitamins and good protein digestibility of acacia. The Atriplex halimus is the only shrub that can be classified as a food very little nutritional value because of the poor digestibility of both its carbohydrate and its caused by its high content of anti-nutritional elements and the large amounts of protein salt it contains. Our work has also confirmed that goats are an animal species able to adapt to the difficult food situation and take advantage of browse low nutritional value in comparison with concentrates and green fodder.

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**Table 1. The characteristic parameters of the gas production of fodder shrubs for each inoculum source (sheep and goat)**

Source of inoculum	shrub forage	A	b	C	Gas 24	Total Gas
Sheep	Acacia cyanophylla	4.5716 <sup>b</sup>	34.72 <sup>d</sup>	0.061 <sup>c</sup>	30.33 <sup>b</sup> ± 3.21	41.0 <sup>b</sup> ± 1
	Atriplex halimus	-0.487 <sup>d</sup>	38.3 <sup>c</sup>	0.053 <sup>d</sup>	27.33 <sup>b</sup> ± 3.21	37.0 <sup>b</sup> ± 3
	Opuntia ficus	6.1301 <sup>a</sup>	56.23 <sup>a</sup>	0.065 <sup>b</sup>	48.66 <sup>a</sup> ± 3.05	63.3 <sup>a</sup> ± 32
	Medicago arborea	2.9177 <sup>c</sup>	53.19 <sup>b</sup>	0.074 <sup>a</sup>	45.33 <sup>a</sup> ± 6.8	57.6 <sup>a</sup> ± 9.3
Pr> F		<0.0001	<0.0001	<0.0001	0.0007	0.0006
Goats	Acacia cyanophylla	4.5426 <sup>b</sup>	42.63 <sup>d</sup>	0.046 <sup>b</sup>	33.33 <sup>c</sup> ± 1.52	47.33 <sup>c</sup> ± 2.30
	Atriplex halimus	1.5222 <sup>c</sup>	48.88 <sup>c</sup>	0.028 <sup>d</sup>	24.66 <sup>d</sup> ± 0.57	45.0 <sup>d</sup> ± 1
	Opuntia ficus	5.4667 <sup>a</sup>	67.97 <sup>a</sup>	0.039 <sup>c</sup>	45.0 <sup>a</sup> ± 2.64	70.33 <sup>a</sup> ± 4.04
	Medicago arborea	1.0383 <sup>d</sup>	61.92 <sup>b</sup>	0.048 <sup>a</sup>	44.0 <sup>b</sup> ± 0	62.33 <sup>b</sup> ± 2.08
Pr> F		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

\* With total -Gas: gas 96h incubation (ml / 300mg DM) - Gas 24: 24 h of incubation gas (ml / 300 mg DM) - a: the gas produced from the soluble fraction (ml / 300 mg DM) -b product from the insoluble but potentially fermentable fraction (ml / 300 mg DM) gas - c: speed gas production (h<sup>-1</sup>)

\* a,b,c and d : Mean in the same column for the same source of inoculum and bearing different letters are significantly different threshold α = 0.05.

**Table 2. Estimated parameters from gas produced at 24 hours of incubation**

Source of inoculum	Shrub forage	ME (Kcal/Kg DM)	DOM (%)	SCFAT(mmol/syringue)
Sheep	Acacia cyanophylla	1750.03 <sup>b</sup> ± 104.48	50.25 <sup>b</sup> ± 2.86	0.664 <sup>b</sup> ± 0.07
	Atriplex halimus	1692.2 <sup>b</sup> ± 104.49	50.03 <sup>b</sup> ± 2.86	0.593 <sup>b</sup> ± 0.07
	Opuntia ficus	2208.52 <sup>a</sup> ± 99.30	63.39 <sup>a</sup> ± 2.72	1.103 <sup>a</sup> ± 0.07
	Medicago arborea	2220 <sup>a</sup> ± 221.25	63.02 <sup>a</sup> ± 6.04	1.023 <sup>a</sup> ± 0.16
Pr> F		0.0024	0.003	0.0007
R <sup>2</sup>		0.82	0.80	0.86
Goats	Acacia cyanophylla	1847.55 <sup>b</sup> ± 49.65	52.917 <sup>b</sup> ± 1.35	0.736 <sup>b</sup> ± 0.03
	Atriplex halimus	1605.52 <sup>c</sup> ± 18.77	47.66 <sup>c</sup> ± 0.51	0.529 <sup>b</sup> ± 0.01
	Opuntia ficus	2089.34 <sup>a</sup> ± 85.99	60.14 <sup>a</sup> ± 2.35	1.133 <sup>a</sup> ± 0.23
	Medicago arborea	2176.57 <sup>a</sup>	61.84 <sup>a</sup>	0.991 <sup>a</sup>
Pr> F		<0.0001	<0.0001	0.0011
R <sup>2</sup>		0.96	0.96	0.85

\* With -ME: metabolizable energy - of OM digestibility MO - SCFAT: Total short chaine fatty acids

\* a,b,c and d : Mean in the same column for the same source of inoculum and bearing different letters are significantly different threshold α = 0.05.

**Table 3. Effect of inoculum source on ammonia nitrogen concentration in N-NH<sub>3</sub>**

Shrub forage	Source of inoculum	N-NH <sub>3</sub> (g/L)
Acacia cyanophylla	sheep	326.67 <sup>a</sup> ± 132.32
	goats	224 <sup>a</sup> ± 48.49 0.2756
Atriplex halimus	sheep	336 <sup>a</sup> ± 28
	goats	140 <sup>b</sup> ± 48.49 0.0037
Opuntia ficus	sheep	270.66 <sup>a</sup> ± 70.46
	goats	140 <sup>b</sup> ± 28 0.0405
Medicago arborea	sheep	494.67 <sup>a</sup> ± 58.29
	goats	214.67 <sup>b</sup> ± 32.33 0.0019

\* Where N-NH<sub>3</sub>: ammonia nitrogen concentration in N-NH<sub>3</sub>

\* a,b,c and d : Mean in the same column for the same source of inoculum and bearing different letters are significantly different threshold  $\alpha = 0.05$ .

### Author' biography



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