Journal of Agriculture and Environmental Sciences
June 2018, Vol. 7, No. 1, pp. 83-92
ISSN: 2334-2404 (Print), 2334-2412 (Online)

Published by American Research Institute for Policy Development
DOI: 10.15640/jaes.v7n1a9
URL: https://doi.org/10.15640/jaes.v7n1a9

The Antioxidant Activity and the Ruminal Fermentation Parameters of Moringa Oleifera L. among Sheep and Goats

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Abstract

Tunisia is a country that is characterized by the diversity of its climate which allows the proliferation of many of its plants that are rich in bioactive substances and that help bring strong values as they can also be used in the fields of animal or human nutrition. In this context, we were interested in going through the physiochemical study and the antioxidant activities as well as the digestibility of the *Moringa oleifera L.* leaves that are refined in the Mornag region. To achieve this, we have created the physicochemical characterization of the plant (DM, MM, OM, CP, and FAT), the parietal carbohydrate content, the quantification of the phenolic compounds and the evaluation of the antioxidant activity of the Aqueous extracted after doing the DPPH test. Our results have proved that the *Moringa oleifera* leaves are rich in the mineral material (9.8 ± 0.06% DM) and in total protein (54.68 ± 0.4). The colorimetric dosage by maceration extraction, and by the use of two solvents, has shown us a change in the total polyphones content in favor of methanol (236.66 ± 1.53 mg EAG / g DM). On the other hand, the distilled water was characterized with a much higher capacity in extraction than that of methanol for the flavonoids. Hence, the study of antioxidant activity has also shown that the *Moringa Oleifera* leaves have a significant antioxidant power (IC50 = 137.65 µg / ml). In fact, the In vitro fermentation by the ruminal microbiota of ruminants has revealed that the total gas production generated by the anaerobic degradation, has the lowest value (30.33 ml) among sheep, while the highest volume (59.16 ml) was to be observed among goats. Similarly to this, the digestibility results of OM, VFA and ME has shown that the studied substrate was more digestible among goats than it was among sheep. These valuable results on Moringa can be used in the field of both pharmacology and animal nutrition because the Moringa leaves represent a feeding source of a high nutritional quality for the ruminants.

Keywords: *Moringa oleifera L.*, secondary metabolites, anti-radical activity, digestibility.

1. Introduction

In the field of animal nutrition, researchers that are made about the diversification of alimentary resources and their animal-feed interaction are currently considered to be one of the most actively researched areas. Indeed, the leaves of *Moringa oleifera L.* (Moringaceae) can be used for the prevention and correction of the malnutrition thanks to their exceptional nutritional properties (Ndong et al., 2007a; Saint Sauveur and Broin, 2010 and Moyo et al., 2011). *Moringa oleifera* is a tropical plant that has multiple uses, it mainly grows in Africa and Asia where it has already been used as a complement in animal feeding (Tedonkeng et al., 2005; Reyes-Sánchez et al., 2006 and Mendieta- Araica et al., 2011).

For a long time, and in spite of the important development achieved in the pharmaceutical industry, the natural remedies and especially the medicinal one, plants used to represent the leading resource of our grandparents medicine. Some of them have been devoted in order to treat different diseases among both humans and animals (Saddiqi et al., 2010), and that is due to the presence of bioactive molecules in it. In fact, several studies have also proved its anti-inflammatory and antioxidant properties (Yang et al., 2006a; Ndong et al., 2007b; Nandave et al., 2009;

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Atawodi et al., 2010; Atawodi 2011 and Moyo et al., 2012). According to Duke (1983), *Moringa oleifera* develops in all types of soil; from acid to alkaline. And at the elevation of the sea level at about 1800 meters; it grows up to be an evergreen tropical tree with deciduous leaves (Pandey et al. 2011). These leaves contain a high amount of vitamins A, B and C (Sanchez-Machado et al., 2010 and Mendieta-Araica et al., 2011a).

Although its richness in minerals, these leaves also do contain some anti-nutritional substances. Among these bioactive compounds, we ought to find total polyphenols (0.67-3.4%), and these only contain negligible amounts (1.4%) obtained by Makkar and Becker (1996) and are undetectable sometimes. They also include oxalate, saponins and phytates as; 4.1%, 1.2% and 3.1% (Gupta et al., 1989).

In addition to this, the *Moringa oleifera L.* plants are also known for their production of a large mass of leaves which represent a feeding source, with a high quality potential, for ruminants (Foidl et al., 2001 and Sanchez-Machado et al., 2010).). Similarly to that, they can also be fed fresh or dried, and after being dried they can be stored for long periods of time without any deterioration within their nutritional value (Foidl and al., 2001).

It is within this frame of work that our work's aim is to nutritionally characterize the leaves of Moringa oleifera L. through a phytochemical analysis, an estimation of antioxidant power by the DPPH test and a study of kinetics of in vitro gas production with the presence of rumen juice from sheep and goats.

2. Material and methods

2.1. Chemical composition

The *Moringa oleifera L.* leaves are mainly harvested from the Mornag field and at an altitude of 26 m whose GPS coordinates are as following ;N: 36 ° .39'45 " and E: 10 ° .16'30'. The leaves are dried in the open air in the dark and are next grinded, using a Retch-type grinder.

All these samples were analyzed for dry material (DM), mineral material (MM), organic material (OM), total nitrogenous material (CP), FAT, and Crude fiber as following the AOAC method (1995). The results of the different chemical parameters are mainly expressed related to the dry material (DM), as for the total nitrogenous content or crude protein content (CP) it can be obtained by measuring the nitrogen (N) which, according to the Kjeldahl method, contains three main stages and these are ; mineralization, distillation, and titration (AOAC, 1990).

Determining the constituents of plant cell walls as: cellulose, hemicelluloses, and lignin is to be done by the method of Van Soest et al. (1994) from the neutral detergent-insoluble residue (NDF), the insoluble residue and acid detergent (ADF).

2.2. Biochemical analyses

2.2.1. Total lipids

Before extracting the total lipids from the sample given, we put them in boiling water from 5 to 10 min in order to inactivate the phospholipids (Benson and Strickland, 1960; Douce, 1964). The extraction is to be achieved according to the method as described by Marzouk (1979) and Marzouk and Cherif (1981). The plant sample is grounded in a porcelain mortar, and the lipids are then extracted with chloroform-methanol in the proportions 2/1 (v / v). The total volume to be used later is 15 ml of solvent per gram of dry material. The mixture is then filtered in tubes of test and placed in an oven at 60 ° C. for 48 hours.

2.2.2. Preparation and obtaining of crude residue

The preparation of the methanolic and the aqueous extracts for determining anti-nutritional substances was achieved following the method of Owen and Johens (1999), which consists first of all in maceration for a period of 24 hours by using a methanol solution (for the methanolic extract) and an aqueous solution (for the aqueous extract). Later on, the secondary metabolites are to be extracted by a maceration of 1 g of powder in 20 ml of methanol with a 20 ml of distilled water for 24 hours. After filtrating it using the filter paper, the filtrate is then evaporated to get dry at 50 ° C. into a ventilated oven.

The dry extracts are weighed and then solubilized with a 3 ml of methanol and a 3 ml of distilled water for the methanolic extract and the aqueous extract, and is then stored at 4 ° C. for the subsequent analyses.

2.2.3 Total Polyphenols

The definition of the phenol compounds was to be extracted through following the method of Singleton and al (1999) and through using the Folin-Ciocalteu reagent (which is a mixture of phosphotungstic acid and phosphomolybdic acid) and an aqueous solution of sodium carbonate. Na2CO3 (20%).

A quantity of 500 µl of the diluted extract (methanolic or aqueous) is then mixed with 500 µl of the Folin-Ciocalteu reagent for ten times. After stirring the mixture, Na 2 CO 3 (20%) is then added. Finally, and after period of 90 min of rest in the dark, the absorbance is to be read at a wavelength of 760 nm. The polyphenols contents are to be expressed in mg of gallic acid equivalent per gram of dry matter (mg EAG / g MS).

2.2.4 Total flavonoids

The flavonoids that are contained within the (aqueous) methanolic extracts of the plants are to be measured through the aluminum trichloride method (Yi and al, 2007). 1 ml of the sample or the standard (prepared in methanol) is added to a 1 ml of the AlCl3 solution (2%). Therefore the mixture gets mightly stirred. And right after 30 minutes of incubation, the absorbance is read at 430 nm.

A calibration curve that is established by quercetin and that is performed under the same operating conditions such as these of the samples will be used for the quantification of flavonoids. These flavonoids contents are to be shown in mg of Quercetin that is a per gram equivalent for dry matter (mg EQ / g MS).

2.2.5 Condensed tannins

In fact, the assessing of the condensed tannin content was determined through the use of a method described by Sun and al (1998). ; a 50 µl of the diluted aqueous sample with a 3 ml of Vanillin solution (4% in methanol) and a 1.5 ml of concentrated H 2 SO 4 are mixed in a test tube. The mixture is then exposed in the dark for 15 minutes, and the absorbance was then measured at a 500 nm. The falconoid contents are also expressed in an mg of Catechin that is equivalent to per gram of dry matter (µg EC / g DM).

2.2.6 Antioxidant activity

According to the method of Ben Ammar et al (2009), a test portion of a 1 ml of the extract at different concentrations (0.125, 0.250, 0.5 mg/ml) is to be placed in the presence of a 1 ml of a methanol solution of DPPH of concentration 0.06 mm. The mixture remains for a period 30 min to rest in the dark for incubation, and the absorbance is then measured at 517 nm using a UV-visible spectrophotometer against control (without any extract). The results that are expressed as percent inhibition (I %) estimated by the equation:

$$I\% = [(Abs\ control - Abs\ test) \div Abs\ control] \times 100$$

IC50: It is the concentration needed in the extract to obtain a percentage of 50% of the reduced form of the radical DPPH

2.3 In vitro production of total gas

The selection of samples of the rumen inoculums were taken from adult sheep and goats immediately after the slaughter at the Tabarka municipal slaughterhouse in northwestern Tunisia. The rumen contents are then homogenized and filtered through four layers of surgical gaze to remove the solid phase. Into the laboratory, the contents of the flask were emptied into an industrial mixer and then purged simultaneously with the CO2 to maintain anaerobic conditions (Grant and Mertens, 1992). After mixing them, the rumen fluid was transferred to a 100 ml glass syringe. The fermentation medium is to be obtained when mixing in each syringe: 10 ml of the filtered rumen juice, 20 ml of an artificial saliva and 300 mg of the ground substrate (3 replicates per sample). This was put in a water bath at 39 ° C, purged with CO2 and continued as it was recommended by Goering and Van Soest. (1970). Thereafter, the syringes were to be shaken in order to make sure they've reached the desired homogeneity, 30 minutes right before the reading is done every 2 hours. The incubation is stopped when the production of gas becomes almost stable.

2.4 Statistical analyses

The parameters of the characteristics of the kinetics of gas production are usually predicted according to the nonlinear regression by the use of the NLN procedure of the SAS (1989) and according to the model of Ørskov and Macdonald (1979).

The equation of the model is as following: Y = a + b (1-e-ct)
With: Y: volume of the gas that is produced after each incubation time (ml); a: production of the gas from the easily fermentable soluble fraction (ml); b: production of the gas from the potentially fermentable insoluble fraction (ml); c: gas production rate (h-1); t: incubation time (h).

3. Results and discussions

3.1. The chemical and parietal composition

The results that are to be presented in Table 1 and 2 show the chemical characteristics and the wall composition of Moringa oleifera L. Indeed, the DM content was of a percentage of 98.5% which is higher than that of the shrubs of the maquis (Selmi et al., 2011) and is comparable to the concentrated food. Our results have also revealed that our plant is rich in mineral material, added to a proportion of about a 9.8%. Moreover, the levels of MAT and total protein were of the order of a 8.75% and a 54.68% .These values are significantly higher than those which were established by Tété-Benissan (2012) .This latter, found a protein content that was equal to 3500 mg / g of powder or 350 mg / g MS. As for the fat content in the present study, it revealed that leaves of Moringa oleifera are high in fat (7.12%) which is higher than the value found by Olugbemi and al. (2010c).The crude protein content of leaves is 54.68% which consequently proves that leaves are an excellent source of protein for livestock food supplies.

Table 1. Chemical composition (% DM) of *Moringa oleifera*

% DM	%MM	%CP	FAT	% C
89.5±0.08	9.8±0.06	8.75±0.06	7.12±0.6	52.3±0.04

DM: dry material; MM: mineral material; CP: Crude protein; C: Carbon

Work that was made on the powders of Moringa oleifera L. showed that the leaves were characterized by a high content of NDF, which represented the total cell walls; and it is a percentage of a 31.66%, ADF (13.15%), lignin (10.11%) and HC of (18.51%). These values are significantly higher than the results that were established by Foidl and Makkar (2001) who found the following proportions NDF (21.9%), ADF (11.4%) and the Lignin of a 1.8%.

The results showed that the latter species contains a high content of crude fiber of (2.91%). The latter also occupies a significant part of the dry material. When the leaves are incorporated at a high level of cellulose, they do not only reduce their total digestibility but also tend to decrease the overall digestibility of the foliar proteins in the alimentary food resources (Gidennt, 1994). The latter has also shown that the increase in the level of fibers in the food supplies is negatively correlated with the digestibility of the dry matter.

Table 2. Wall composition of leaves of *Moringa oleifera L.* (% DM)

CF	NDF	ADF	LIG	FS	HC
2.91±1.28	31.66±1.97	13.15±0.43	10.11±0.86	68.5±1.97	18.51±1.67

CF: crude Fiber; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; LIG: lignin; HC: Hemicelluloses FS: Soluble fraction.

3.2. Biochemical analyses

The results of the biochemical analyzes (total lipids, total polyphenols, flavonoids and condensed tannins) are presented in Table 3. The *Moringa oleifera L.* leaves contain a high content of total lipid of 7.21 ± 1.1 mg / g DM. This result was to be an agreement with El Sohaimy et al (2015) who both found a value that was equal to 7.76 ± 0.21 g / 100g DM.

As for the anti-nutritional factors, they are substances that are naturally present in fodder trees and shrubs; they are also known for reducing the nutritional quality and valorization of these shrubs by animals, regardless of their nutrient content. The analysis of the secondary metabolites was to be done on methanol and aqueous extracts. In fact, the total polyphenols were 236.66 ± 1.53 mg EAG / g DM and 130.83 ± 3.69 mg EAG / g DM respectively for the methanol and aqueous extracts. Our results are higher than those obtained by Sreelath and Padma (2009) who found a low level of phenolic compounds which was of the order of 28.02 mg EAG / g DM and 45.41 ± 0.02 mg EAG DM/ml respectively of the aqueous and methanol extract. These variations ought to be explained by the pedo-climatic conditions. The presence of these phenolic compounds, and according to some authors (Frutos et al., 2002 and Khanal and Subba., 2001), could have had a limited effect on the availability of nutrients from forages for the ruminal microbiota.

In ruminants, different types of phenols including the simple phenols and polyphenols, actually do affect the nutritional value of aliments (Ahn et al., 1989). In addition to that, their effects can be explained in multiple aspects: modification of rumen microbiota composition, inhibition of microbial enzymes, complexation with aliments' proteins, polysaccharides, and minerals. They may also transmit with their metabolic products and turn into tissues, Hence, cause toxicity (Cheekeet, 1995).

In addition to that, the colorimetric dosing of the flavonoids that were expressed in milligram equivalent quercetin per gram of a dry material, have showed that the Moringa oleifera L. leaves collected in the Mornag region contained a high content for the two solvents that were previously used, and which are 77. 33 ± 3.76 mg EQ / g DM for the methanol extract and 86.44 ± 4.55 mg EQ / g DM for the aqueous extract. This variation is to be confirmed by Sreelath and Padma (2009) who mentioned that the flavonoid content lightly depends on the type of solvent, which discerned a 28.0 mg EQ / g DM for the aqueous extract and a 27 ± 0.03 mg EQ / g DM for the methanol extract.

Concentrations of condensed tannins in our plant sample also varied in contribution to the solvent and were 22.52 ± 1.02 µg EC / g DM and 27.79 ± 0.68 µg EC / g DM respectively for methanol and aqueous extracts. We ought to deduce a little different for the two excerpts, those which; converges with those which were identified by Makkar and Becker (1996), and those which were to be obtained negligible amounts of tannins (1.4%). Additionally, they have a very high antibacterial power (Bassene and al., 1995).

The levels of the total polyphenols and flavonoids also varied according to the region and pedo-climatic factors. Indeed, Iqbel and Bhanger (2006) have shown a spatial-temporal variation in the overall polyphenols content and flavonoids, as they found within the regions of Pakistan, that during December the total polyphenols content variations are 9, 17g /, 100g in Nawabshah, 13.09g / 100g in Mardaan, 12.39g / 100g in Balakot and the highest value of (13.56g / 100g) was found in Mardaan during March.

Table 3. Variation in the total Polyphenols, Flavonoids, Condensed Tannins and Total Lipids

	PT	FT	TC	LT
Methanol extract	236.66±1. 53	77.33±3.76	22.52±1.02	7.21±1.1
Aqueous extract	130.83±3.69	86.44±4.55	27.79±0.68	

P T: Total Phenols (in equivalent mg Gallic acid / g DM); FT: Total Flavonoids (in equivalent mg Quercetin / g DM, TC: Condensed Tannins (in equivalent µg Catechin / g DM); TL: Total Lipids

3.3. Antioxidant activity using the DPPH test

The antioxidant activity of aqueous extracts of Moringa oleifera L. was evaluated in vitro by the DPPH test, and the result was expressed in terms of percentage reduction of DPPH. The result obtained is represented in FIG. 1, which illustrates the effectiveness of the Moringa extract in trapping the DPPH radical, translated by the inhibition rate (I%) as a function of the different concentrations; the evolution of the anti-radical activity is dose-dependent because it increases with the increase of the levels of the extracts in the reaction medium.

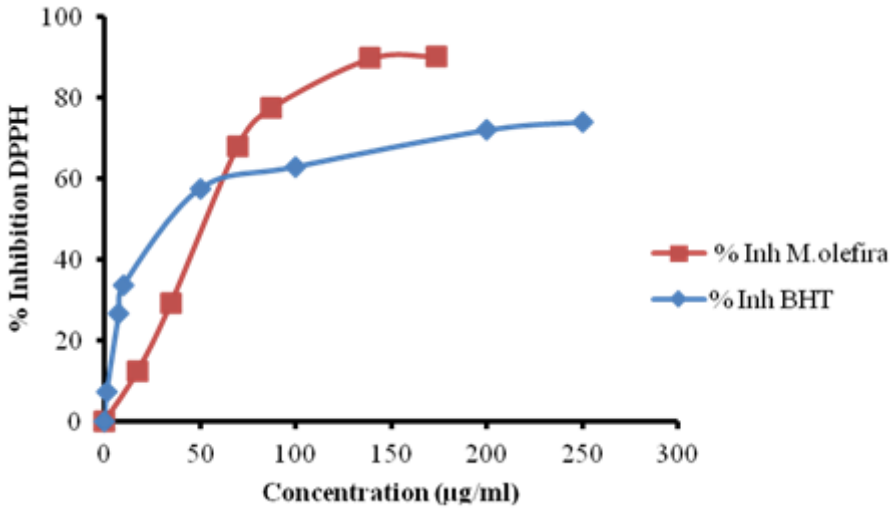

Figure 1. Curve of the anti-radical activity of the aqueous extract of Moringa and the BHT against 2, 2-diphenyl-1-diphenyl-1-picrylhydrazyl (DPPH)

Through this result, one can deduce that the aqueous leaf extract of Moringa has exerted an excellent activity respectively to the DPPH radical which reaches a 90% inhibition at the concentration of 180 µg / ml, and which is usually higher than that of the synthetic antioxidant which is BHT (73% inhibition at 250 µg / ml).

Moringa oleifera L. is to be known for its antioxidant power and its interest in fighting against free radicals. This can be explained through the contribution of phenolic compounds that are present in the Moringa leaves and because they have an IC50 equal to 48 , 65 ± 0.1 µg / ml, similar to this, El Sohaimy et al (2015) found out an IC50 closely related to our result (46.77 ± 0.13 µg / ml) and for the aqueous extract contrary to what they found with IC50 the results were lower of those of the present study and which were 33.11 ± 0.08 µg / ml for the methanol extract (70%) and 44.10 µg / ml for the ethanol extract (70%). Thus, that the antioxidant power depends on the type of solvent.

These results indicate that most of the antioxidant plant activities do correlate with total polyphenols and flavonoid levels, which plays an essential role in defending free radicals that are consistent with data from the different authors (Wang et al., 2009 and Dell 'Agli et al., 2004). In addition, the chemical compounds of Moringa found by HPLC are a total of isothiocyanates and astragalin which have antioxidant activities (Engsuwan et al., 2017). Gaafar et al (2016) showed that the antioxidant power is correlated with the richness of the plant in phenol compounds which are ellagic acid (41.1%), quercetin (6.3%) and benzoic acid (26.01%).

3.4. Kinetics of gas production

The production of gas depends mainly on the degradation rate and the nature of the characteristics of carbohydrates within the aliments. It can also change from one ruminal environment to another depending on the animal species as it is shown in Figure 2.

Our results showed that the gas volume at 24 h incubation significantly varies ($p < 0.01$) between the two animal species. The gas volume among sheep has the lowest value (30.33ml), while the highest gas volume (59.16 ml) was to be noticed among goats. This can be explained by the presence of high content of MAT. In this context, Arhab (2000) shows that the In vitro digestibility is positively correlated with the nitrogen content. In addition to this, the rumen goats happened to seem more buffered and contained more bacteria and protozoa than that of the sheep because they have specialized protozoa that are called type B (Epidinium and Eudiplodinium), and that is more effective in the degradation of cellulose which converges with those of Rouissi (1994) and Jouany (1989). The kinetics of gas production among goats and sheep follows a first-order exponential model, among goats the gas volume was 73.39 (1 e-0.07t) whereas, among sheep, it was 51, 64 (1- $e^{-0.03t}$).

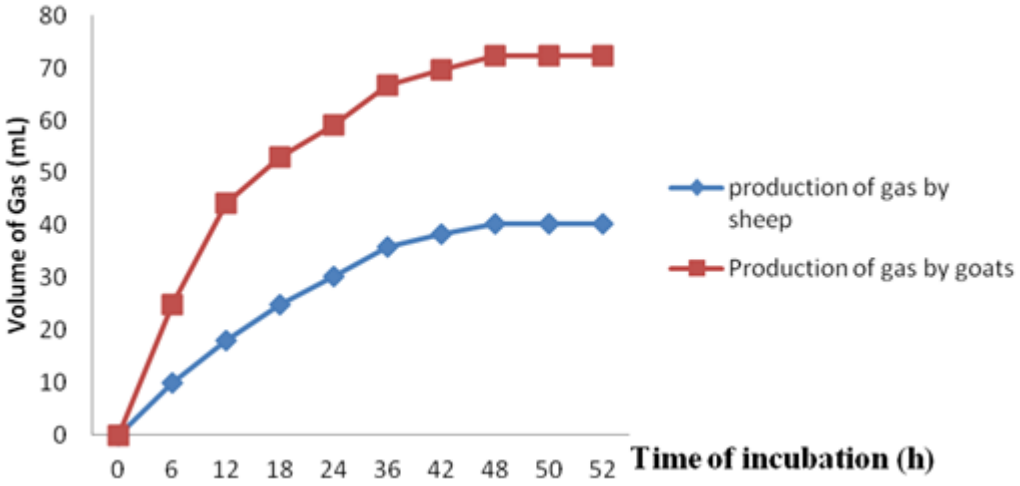

Figure 2. Gas production kinetics curve after the incubation of *Moringa oleifera L.* according to the animal species.

3.5 The ruminal fermentation parameters of M. Oleifera L. according to the animal species

The digestibility of OM, metabolizable energy (ME) and the total volatile fat acid (DTFA) concentration are summarized in Table 4. In fact, the studied shrub "Moringa" has statistically different digestibility values ($p < 0.01$) in both animal species. Indeed, among goats, the metabolizable energy and total VFA content are the highest, but on the other hand production among sheep shows a minimum content, for example, the content of ME is of the order of 11 and 7 Kcal/kg of DM respectively for goats and sheep. This can be explained by the good digestive use of Moringa among goats, which later results in intense gas production. Similarly, the much higher value of Digestibility OM is reported among goats, while sheep have low values considered of 74.5 ± 0.92 and 48.86 ± 0.51%. This may be thanks to a better ruminal microbial growth among goats.

It is concluded therefore that volatile fat acids, which represent the source of energy for ruminants, are the ending products of ruminal digestion and which include multiple compounds extracted from plant cell walls, such as cellulose, hemicelluloses, and pectins. Or cellular content such as soluble sugars. In addition, their concentration depends on the amount of energy that is provided by the food and the activity of microorganisms in the rumen. (Cuveleir et al., 2005).

Table 4. Variation of fermentation parameters of the Moringa rumen by animal species.

	DOM (% DM)	ME (kcal/kg DM)	TVFA (mmol/syringe)
sheep	48.8^{b}	**7**^{b}	0.66^{b}
goats	74.5^{a}	11^{a}	1.35^{b}
Species effect	**	**	**

4. Conclusions and Recommendations

Moringa oleifera L. contains a significant content of mineral matter and bioactive substances. In fact, it is endowed with a considerable antioxidant power. Our results have shown that the plant is constituted of essential resources that can effectively contribute to the nutritional needs of small ruminants conducted extensively. Moringa is a high-quality potential alimentary source for ruminants, as it has significant digestibility for both animal species. We propose to succeed in the cultivation of this vital plant so that it would become widespread in Tunisia because it is endowed with an interesting power of adaptation. On the other hand, a pharmacological study and an evaluation of the anti-inflammatory and anti-diabetic effects were proved to be effective in order to carry out the characterization of this plant's species.

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