Journal of Applied Biological Sciences
E-ISSN: 2146-0108 17(1): 46-54, 2023
DOI: 10.5281/zenodo.7579713
Research Article

ASSESSMENT OF VOLATILE FATTY ACIDS AND METHANE PRODUCTION *IN VITRO* OF *OPUNTIA FICUS-INDICA* CLADODES IN AN ALGERIAN ARID AREA

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(Received 06th May 2022; accepted 09th October 2022)

ABSTRACT. The main objective of the present study was to evaluate the volatile fatty acids (VFAs) and methane (CH_4) production of cactus *Opuntia Ficus-Indica* (*O. ficus indica*) Cladodes, *Medicago sativa* L. (*M. sativa*) and barley straw (*H. vulgare*) using chemical composition and *in vitro* gas production. There were significant ($p<0.05$) differences among studied samples in all measured nutrients except dry matter (DM) value. *O. ficus indica* showed particularly the highest Ash content (284.07 g/kg DM). The crude protein (CP) of the studied feedstuff ranged from 51.41 to 156.09 g/kg DM and it was especially high within *M. sativa* and low in *H. vulgare*. No significant difference was observed in pH values, total VFAs, propionate concentrations and CH_4 production (ml/100 gas) among plants species studied. The lowest Acetate concentration was observed in *H. vulgare* (711.17 mmol/mol) and *M. sativa* (715.33 mmol/mol), respectively ($p < 0.05$). The maximum *in vitro* gas production was recorded with *M. sativa* and *H. vulgare.* Based on the results, it can be concluded that *O. ficus indica* could replace common plants species, especially in the arid and semi-arid areas in which livestock production frequently suffers from low efficiency and big losses. Furthermore, these plantations in agroforestry development could consolidate the protection of the environment against erosion, allowing the fixation of the soil and its protection against water erosion.

Keywords: *In vitro gas production, Opuntia ficus indica, rumen fermentation, methane production, volatile fatty acids.*

INTRODUCTION

Due to low feed quality and availability, the majority of ruminant production systems have deficient productivity, mainly in the arid and semi-arid areas. High demands of food accompanied with a decrease in crop yields are high-pressuring farmers of the eastern and western steppe land to develop further land cultures at the expense of grassland and browses [1, 2, 3]. Ruminant animals have the ability to ferment and digest most of the lingo-cellulosic feed components in order to gain their maintenance and production energy requirements. In addition, they are capable of synthesizing rumen microbial proteins with the use of some non-protein nitrogen sources. However, during the fermentation process occurring inside the largest digestive compartments some methanogen archaea, in order to acquire authotrophically their energy, convert hydrogen molecules and carbone dioxide to produce potent greenhouse gas methane. It is estimated that, the enteric methane produced fundamentally by cattle, then sheep and goats, provides approximately 30% of the earth atmosphere methane discharges, conducting the livestock production sector to be considered one of the largest anthropogenic sources on the globe [4, 5]. *Opuntias* have been known remote time for their wide-ranging uses and properties and they are found in multiple lands. Prickly pear (*Opuntia ficus-indica*) plantations are a valuable investment for some arid and semi-arid regions around the world [6]. Commonly, unripe cladodes of prickly pear and the fruit are used as human nourishment [7, 8]. However, mature stems or cladodes can be used to feed animals [9]. The Algerian steppe including M'sila conceals in its folds a diversity of biological resources, animal and biomass resources which constitute a real cradle of sheep breeding. *Opuntia ficus-indica* plantations are a valuable investment for some arid and semi-arid regions around the world. Habitually, fruits and unripe cladodes of prickly pear are utilized as human nourishment. Conventional waste control project for *Opuntia Ficus-Indica* incorporate its application as ruminant feed or in the cosmetics industry. In addition, they are used as additives in the manufacture of shampoo, astringent lotions, and creams, humectant soaps, moisturizing gels for face and hair softeners [10]. *Opuntia ficus indica* may represent a complementary source of fodder, especially during periods of drought [11, 12]. Forage cacti are rich in digestible carbohydrates, lipids, vitamins, water, minerals and low in protein [13]. The present study aims to determine the volatile fatty acids and methane productions of *Opuntia ficus-indica* cladodes in an arid area of Algeria, using the *in vitro* gas production technique, versus the whole plant of alfalfa and barley straw used as standards.

MATERIALS AND METHODS

Study Area and plants species

M'Sila province is located 240 km south-east of Algiers, in the country's highlands with latitude 35◦18' and 35◦32' North and 4◦15' and 5◦06' East, Winter is the coldest season with noticeable low and rare rainfall estimated between 100 to 250 mm/year and a very hot and dry summer [4]. Average annual temperatures ranged between 13 °C to 23 ◦C with extremes of -5 ◦C in winter to 46 ◦C in summer [14]. The state is made up of 15 Daïras (districts), and the most populous city is M'Sila, come after Bousaada, familiar as the "gateway to Sahara" (Fig. 1) [15].

Three plants species were used in this study which are the inermal cactus (*Opuntia ficus indica* f. inermis) cladodes, whole plant alfalfa (*Medicago satifa* L.) and barley straw (*Hordium vulgare*). The choice of these species was based on their large cultivation area, high availability, palatability for ruminants and their capacity to produce biomass in conditions of absolute marginality of the soils, thus constituting a food resource for livestock in arid conditions.

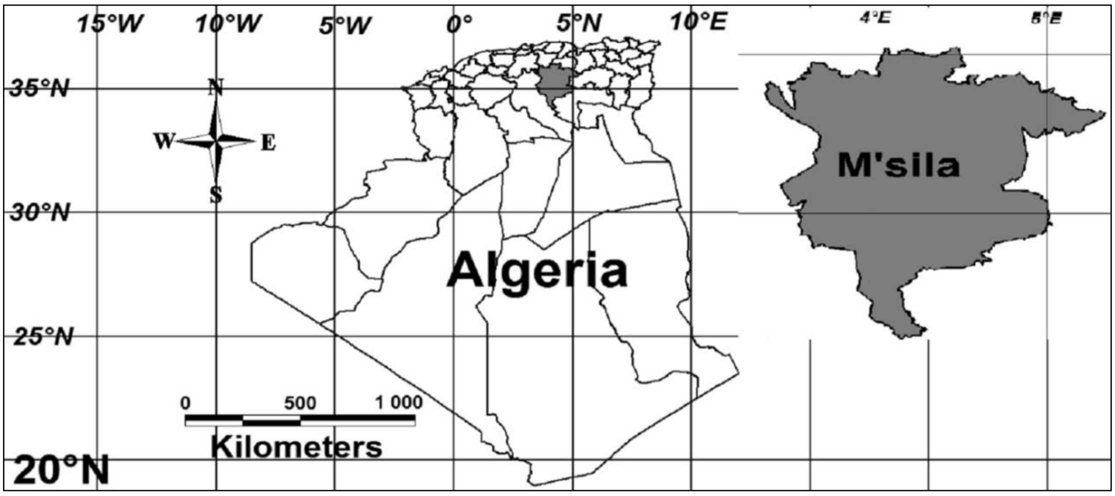

***Fig. 1**. The study area.*

Chemical analysis

AOAC [16] methods and milled plants samples were utilized to evaluate different parameters such as: dry matter (DM), acid detergent fiber (ADF) and neutral detergent fiber (NDF) and crude protein (CP). Every chemical analysis was achieved threefold. Both ADF and NDF fibers contents were analyzed with the use of an apparatus (ANKOM 200 Fiber Analyser, ANKOM Technology, Macedon, NY, USA) ANKOM brand using 0.5 g samples [17]. These two determinations were made using different samples and each sample was analyzed threefold.

In vitro determination of volatile fatty acids and methane production

A short-term incubation batch was managed to determine the *in vitro* VFA composition and methane production of studied plant species following Menke and Steingass [18]: diluted solution (1: 4 v/v) containing rumen liquor mixed with bicarbonate buffer, resazurin and macro and micromineral solutions was prepared. Three adults rumen cannulated Merino ewes (mean mass 48.40±3.33 kg) were utilized to obtain rumen liquid. The experiment animals were maintained in cages, nourished alfalfa hay *ad libitum* (ADL 71g, ADF 355 g, NDF 502 g, and CP 167 g/kg DM) under open access to mineral/vitamin block (NUTRI-FOS 15; Phosphorus + Calcium + 15% NPN) and water. Then, each serum bottles receives 50ml of diluted ruminal fluid 10ml of rumen juice and 40ml of artificial saliva, prepared according to Menke and Steingas [18]. The bottles are then closed with butyl stoppers and crimped with an aluminium cap. The vials are shaken and placed in the incubator at 39 °C for 24 hours. The incubations are carried out in three series using three different rumen juices. Each rumen juice comes from two animals. Each series is performed in two repetitions (two bottles/substrate/rumen juice). That is 6 repetitions for each sample. In order to make the necessary corrections, control vials are also incubated. For this, six vials containing only liquid rumen inoculum are incubated to be used to determine the gas production in the absence of substrate and use it to compensate the flasks containing the substrate studied. After 24h of incubation, the flasks are placed in a water bath set at 39 °C to measure the gas pressure using a pressure meter equipped with a probe (Delta Ohm DTP704-2BGI, Herter Instruments SL, Barcelona). A system of graduated syringes with a valve is used to measure the pressure in millilitres and, on the other hand, to allow the taking of a gas sample for chromatographic analysis to determine the volume of methane produced.

Afterwards, the bottles are placed in an ice bath to stop the fermentation and are opened to measure the pH of their contents with a pH meter (pH meter HI 4521-02). After cooling and in 2 ml Ependorff tubes, previously 0.5 ml of the internal standard solution and 0.8 ml of the rumen juice solution are introduced and stored in the freezer for subsequent VFA determination.

A Perkin-Elmer Autosystem XL GC and Shimadzu GC-14 B GC gas chromatography were used to evaluate individual VFAs concentrations and methane contents in the fermentation gas respectively [19].

Statistical analysis

Every result was examined with the use of one-way analysis of variance [20] and Tukey's test in multiple means comparison ($p<0.05$). SAS software package [21] was used to perform an analysis of variance between different variables.

RESULTS AND DISCUSSION

As presented in Table 1 there were significant ($p<0.05$) differences among studied samples in all measured nutrients except DM value. *O. ficus indica* showed the highest Ash content (284.07 g/kg DM), whereas the lowest concentrations were observed in *H. vulgare and M. sativa with* 71.85 and 90.27 g/kg DM, respectively. The CP of the studied feedstuff ranged from 51.41 to 156.09 g/kg DM and it was particularly high within *M. sativa* and low in *H. vulgare*. *O. ficus indica* showed low value with 79.04 g/kg DM. Higher cell wall components concentrations were observed in *H. vulgare* than in *M. sativa* and *O. ficus indica*. The NDF and ADF contents ranged respectively from 325.93 to 668.17 g/kg DM and from 119.94 to 361.52 g/kg DM, with the highest values for *H. vulgare*. The same trend was observed for CEL and HMC contents with values ranging from (100.82 and 311.98 g/kg DM and 197.90 and 326.10 g/kg DM respectively. *M. sativa* showed the highest ADL content (76.94 g/kg DM), whereas the lowest concentrations were observed in *O. ficus indica* (19.45 g/kg DM).

Table 1. *Chemical composition (g/kg dry matter) of studied samples*

	Plants species				
	O. ficus indica	***M. sativa***	***H. vulgare***	**S.E.M**	**Significance**
DM	949.18	939.18	939.74	17.953	
ASH	284.07^{a}	90.27^{b}	71.85^{b}	61.691	***
CP	79.04^{b}	156.09^{a}	51.41^{c}	6.913	***
NDF	325.93^{c}	470.92^{b}	668.17^{a}	208.468	***
ADF	119.94^{c}	303.16^{b}	361.52^{a}	22.731	***
ADL	19.45^{c}	76.94^{a}	50.88^{b}	1.447	***
CEL	100.82^{c}	227.22^{b}	311.98^{a}	10.456	***
HMC	235.40^{b}	197.90^{c}	326.10^{a}	47.069	***

DM*: Dry matter %,* ***CP****: Crude protein,* ***NDF****: Neutral detergent fiber,* ***ADF****: Acid detergent fiber,* ***ADL****: Acid detergent lignine,* ***CL****: Cellulose,* ***HMC****: Hemicellulose;* ***S.E.M****: standard error mean;* $^{a, b, c}$ *Line means with common superscripts do not differ* ($p<0.05$).

pH value, total VFA, molar portions of VFA, C_2 to C_3 ratio, and CH4 production were presented in Table 2. No significant difference was observed in pH values, total VFAs, propionate concentrations and CH_4 production (ml/100 gas) among plant species studied herein ($p<0.05$). The lowest Acetate concentration was observed in *H. vulgare* (711.17 mmol/mol) and *M. sativa* (715.33 mmol/mol), respectively ($p<0.05$). The concentration of Valerate (18.67 mmol/mol) in the culture medium was highest when *M. sativa* whole plant was incubated ($p<0.05$), whereas the highest butyrate concentration (59.65 mmol/mol) and (63.12 mmol/mol) was observed in *H. vulgare* and *M. sativa* respectively ($p<0.05$). The C_2 to C_3 ratio, equal 3.06, was lowest in *H. vulgare* and *M. sativa* (3.35) and highest in *O. ficus indica* **(**4.99). The maximum total *in vitro* gas production was recorded with *M. sativa* and *H. vulgare* (119.32 and 110.89 ml/g DM) respectively.

Table 2. *Total VFAs production (mmol VFA/g dry matter incubated), molar proportions (mmol/mol total VFA) of Ace, Pro, But and Val at 24 h of incubation, total gas production 24 h and methane production (ml/g dry matter incubated).*

	Plants species				
	O. ficus indica	***M. sativa***	***H. vulgare***	**S.E.M**	**Significance**
pH	6.55	6.47	6.56	0.0188	***NS***
Total VFAs (TVFA)	2.04	2.96	3.17	0.029	***NS***
Acetate (Act)	755.67 ^{a}	715.33 ^{b}	711.17^{b}	489.033	*******
Propionate (Pro)	183.00	190.50	209.67	417.388	***NS***
Butyrate (But)	43.48 ^{b}	63.12 ^{a}	59.65 ^{a}	15.766	*******
Valerate (Val)	7.03 ^{b}	18.67 ^{a}	7.15 ^{b}	1.845	*******
Acetate / Propionate (C_2:C_3)	4.99 ^{a}	3.35 ^{b}	3.06 ^{b}	0.229	*******
Gas production (ml/g DM)	83.83 ^{b}	119.32^{a}	110.89^{a}	82.896	***
CH_4 (ml/g DM)	15.92 ^{b}	25.80 ^{a}	20.14 ^{ab}	23.886	***
ml CH_4/100 ml gas	19.13	21.69	18.25	0.018	**NS**

S.E.M*: standard error mean;* $^{a, b, c}$ *Line means with common superscripts do not differ* ($p<0.05$). *NS: Non significant? ***: Significant*

It is well known that chemical composition of forages influences directly on digestibility and in vitro fermentation, and thus the amount of end-products particularly VFAs released. In the present study, there were significant differences among studied samples in almost all measured nutrients. This difference in chemical components between plant families was anticipated because nutrients built up in plants are affected by genotypic factors (genus) [22]. The CP contents of the plant species studied in our experiment were within the range of reported values in previous literature [23, 24, 25]. The CP contents were always above the minimum content of 70-80 (g/kg DM) required for adequate rumen function and feed intake of ruminant [26]. Due to the relatively high CP content of *O. ficus indica*, this feed resource can be considered a suitable supplement for crop residues such as poor-quality natural grasses (with low N content), straw and shrubs. *O. ficus indica* showed the highest ash content than the two other samples which depends on soil, plant species, and the management factors [27]. This mineral content is much higher than that reported for some legumes and grasses forages by Medjekal et al. [19]. High cell wall components concentrations were observed in *H. vulgare* than in M. sativa and *O. ficus indica*. This significant difference may be explained by some internal morphological or anatomical differences in relation to the cell wall structure and inflexibility [28] and leaves to twigs proportion in the used samples in our experiment. Crop remainders are important roughage sources for ruminant animals, in particular where grassland is limited. *H. vulgare* is typically high in fibre and low in crude protein, although, their inclusion in dairy diets can be a helpful nutrition management tool for livestock production [29]. Furthermore, in order to improve fibrous feed digestibility, some exogenous fibrolytic enzymes are of growing interest as additives to ruminant nutrition [30]. Gas production was higher in *H. vulgare* and *M. sativa* ($p < 0.05$), compared to *O. ficus indica* which agrees with cell wall concentration. Indeed, gas production is closely associated with the amount of fermentable substrate in the diet. These results are in good agreement with other studies which have shown significant differences in gas production at 24 hours between the four studied samples, including *O. ficus indica*, Vetch-Oat hay, *Acacia nilotica* and *Acacia saligna* [24].

Bacterial attachment in the rumen is mainly affected by ruminal pH [31]. In this experiment, no significant differences were observed in pH values and the pH observed was within the limitations allowing high fibre degradation [32]. The pH of the ruminal environment is the result of acid production, salivary buffers and the ration's own buffers [33]. Although it is difficult to define a precise range with certainty, it is generally accepted that under normal rumen conditions, the pH values ranged between 6.0 and 7.0 [4]. VFAs play a key role in supplying energy to ruminants. Their production in the rumen indicates clearly the carbohydrate degradation patterns by rumen microorganisms. Depending on total VFA, acetate and propionate concentrations, *O. ficus indica* seemed to be the high fermentable substrate. In ruminants, VFAs contribute to about 70% of the energy requirements of animals [34] and most of them are absorbed directly through the rumen wall. When Propionate is directed to the liver where it is the main substrate for new gluconeogenesis, its concentration is not limited and it is largely available [35]. These results confirm those obtained previously and relating to both VFAs and methane production [4, 24]. Moreover, changes in the molar proportion of VFAs, accompanying the decrease in methane production, indicate a change in the specific fermentation profile. As shown in Table 2, *O. ficus indica* fermentation produced a high ratio of acetate to propionate and the most likely explanation is the excess acetogenic fermentation due to the substrates rich structure in carbohydrate degradation with high fobrolytic bacterial activities [36].

The higher proportion of acetic acid and higher propionic acid observed in this experiment are in good agreement with other studies with *Prosopis cineraria* leaves [37]. Methanogenesis, process through which methanogens produce methane. Previous research has documented the inhibition of food metabolizable energy capacity and feed energy loss of 2 to15% because of CH_4 production [38]. Furthermore, in recent years, the contribution of greenhouse gas emissions from the livestock system to the atmosphere has become extremely important. Just behind carbon dioxide as an anthropogenic greenhouse gas, CH_4 comes in second place and conduces to aggravate problems of climate change and global warming [39]. The quantity of CH_4 produced by ruminant hangs on the quantity of feed intake [40], and the higher the feed intake, the different the rate of energy consumption lost due to the decrease in CH_4. This effect is partly the result of increased rumen passage and partly the result of the type of VFA manufactured. In addition, CH_4 production increases approximately in proportion to feed intake, but CH_4 emissions/unit animal production decreases when animal productivity improvement is achieved [41]. Likewise, CH_4 production is a unique procedure during rumen fermentation, and eliminating or reducing its production is considered a modern challenge in the livestock production sector. The focus of recent research has been on some plant species secondary metabolites and their antimethanogenic effects. Major interest and investigation into the interpretation of such results could allow future research to identify the chemical components specifically charged with their antimethanogenic activity [42].

CONCLUSION

Based on the results, it can be concluded that *O. ficus indica* is suitable as feed resource that could replace common plant species especially in the arid and semi-arid areas in which livestock production frequently suffers from low efficiency and big losses. Hence, a combination of such feeds having high cell wall component potential and bioactive rich compounds can be a sustainable option to enhance productivity without any adverse effect on the environment. *O. ficus indica* makes a valuable contribution to the food security and nutrition of people throughout the world wherever water is scarce.

Conflict of Interest. The authors declared that there is no conflict of interest.

Authorship Contributions. Concept: S.M., F.Z.B., Design: S.M., F.Z.B., Data Collection or Processing: S.M., Analysis or Interpretation: S.M., Literature Search: S.M., Writing: S.M., S.L.

Financial Disclosure. This research received no grant from any funding agency/sector.

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