**In vitro CULTURE OF RUMEN CILIATE PROTOZOA BASED ON Parmentiera aculeata Kunth MEDIUM**

[CULTIVO in vitro DE PROTOZOARIOS CILIADOS DEL RUMEN EN UN MEDIO A BASE de Parmentiera aculeata Kunth]

Alejandro Ley de Coss, Juan F. Aguirre Medina*, Consepción Arce Espino, Willians de León de León, Saúl Posada Cruz, René Pinto Ruiz, Francisco Guevara Hernández, Susana G. Gayón Amaro

1Faculty of Agronomic Sciences, Campus IV, Autonomous University of Chiapas (UNACH), Coast Highway and Station Huehuetán Railway, Huehuetán, Chiapas, Mexico. CP. 36670. Email: juanf56@prodigy.net.mx.
2Master in Tropical Agropecuary Production Student At UNACH.
3Faculty of Agronomic Sciences, Campus V, UNACH. Villaflores, Chiapas, Mexico.
4Faculty of Veterinary Medicine and Zootechny, Autonomous University of the State of Mexico, Toluca, Estado de Mexico.
*Corresponding author

**SUMMARY**

The present research aims to measure the ciliated protozoa concentration, pH, the concentration of volatile fatty acids (VFA) and the in-vitro dry matter degradation in a culture environment based *Parmentiera aculeata* Kunth. The treatments were: T1, 300 µL of *Avena sativa*; T2, T3 and T4 had 150, 300 y 450 µL of *P. aculeata* Kunth, respectively; and T5, 300 µL of *Chenopodium ambrosioides* L. Every treatment was inoculated with ruminal fluid and incubated for 3, 6, 12, 24, 48 and 72 hours to 38±0.5°C. Based on a randomly experimental design, the data was analyzed by GLM and the means were compared with Tukey (p≤0.05), while the protozoa concentration was analyzed with a rank test. There were differences in the protozoa concentration and pH between treatments; T3 and T4 had the highest amounts (p≤0.05) compared with the other treatments, especially with T5. There was no difference (p≥0.05) in the acetic acid concentration between T1, T2 and T3 compared with T4 at 72 hours and with T5 from 6 hours of incubation, besides for the concentration of butyric acid T5 had the lowest amount (p≤0.05) at 72 hours. The smallest concentration of total VFA was for T4 and T5 from 48 hours. *P. aculeata* Kunth used like subtract helps to preserve for 3 days a ciliated protozoa population of 10^4 without alterate the fermentation process.

**Keywords:** Agroforestry; Livestock; Animal nutrition; Rumen protozoa.

**RESUMEN**

El presente estudio se realizó para medir la concentración de protozoarios ciliados, el pH, la concentración de AGV y la Degradaclón in vitro de la materia seca en un medio de cultivo a base de *Parmentiera aculeata* Kunth. Los tratamientos fueron: T1, 300 µL de *Avena sativa*; T2, T3 y T4 fueron 150, 300 y 450 µL de *P. aculeata* Kunth, respectivamente; y T5, 300 µL de *Chenopodium ambrosioides* L. (planta desfaunante). Cada tratamiento se inoculó con fluido ruminal y se incubaron durante 3, 6, 12, 24, 48 y 72 h a 38±0.5°C. En un diseño al azar; los datos se analizaron con GLM y las medias se compararon con Tukey (p≤0.05), mientras que la cantidad de protozoarios se analizaron con la prueba de suma de rangos. Hubo diferencias en la concentración de protozoarios ciliados y el pH entre los tratamientos, T3 y T4 tuvieron las mayores cantidades (p≤0.05) en comparación con los otros tratamientos, especialmente con T5. No hubo diferencia (p≥0.05) en la concentración de ácido acético entre T1, T2 y T3 con respecto a T4 a las 72 h y con T5 después de 6 h de incubación, además, para ácido butírico, el T5 tuvo la menor concentración (p≤0.05) a las 72 h. La menor concentración de AGV totales fue para T4 y T5 desde las 48 h. *P. aculeata* Kunth en medio de cultivo permitió conservar durante 3 días una población de 10^4 protozoarios ciliados, sin alterar el patrón de fermentación.

**Palabras clave:** Agrosilvicultura; Ganado Vacuno; Nutrición Animal; Fauna ruminal.
INTRODUCTION

In tropical and subtropical regions of Mexico and Central America are many species of forage plants (FP) which can increase the amount and availability of some of the nutrients of a ruminant diet (Sosa et al., 2004). In previous studies with FP the balance energy/protein, the presence and consequences of anti-nutritional factors (saponins, tannins, alkaloids, phenols, etc.) in ciliate protozoarian populations, the effect in the voluntary intake, palatability, digestibility of dry matter and the animal response has been shown (Sosa et al., 2004).

The protozoarian species and its ruminal concentration is related with the nature of the diet, physiological conditions of the animal and the nutritional stress (Göçmen et al., 2001); in addition of the time of the sample and the circumstances associated with the in-vitro process. Because of all this factors the amounts of ciliates that had been reported showed a variation between $10^3$ to $10^9$ cells mL$^{-1}$ of the culture environment or ruminal fluid (Ley de Coss et al., 2011; Kumar et al., 2009). Concentrations greater than $10^4$ ciliates per mL has been found in environments where the energy source was the Avena sativa L flour (Ley de Coss et al., 2011a; 2011b), Manihot esculenta Crantz, Musa spp L., Oryza sativa L. (Coleman, 1981), Triticum aestivum L. hay (Michałowski et al., 2001) and Dactylis glomerata L. while the bacterial population was regulated ($<10^8$ cells mL$^{-1}$) by the addition of antibiotics against gram negatives (2,000 U mL$^{-1}$ penicillin and 130 U mL$^{-1}$ streptomycin) (Dehority, 2008). By other side, the positive effect of the addition of minerals based in K$_2$HPO$_4$, KH$_2$PO$_4$, (NH$_4$)$_2$SO$_4$, NaCl, MgSO$_4$, CaCl$_2$:H$_2$O, Na$_2$CO$_3$: 8%, L-cystein (dissolved in 2N NaOH) + Na$_2$S·9H$_2$O with oxide-reduction indicators like resazurin and CH$_3$COONa 1.5%; to the environment was evaluated (Dehority, 2008; Ley de Coss et al., 2011a; 2011b). Therefore, the objective of this study was to evaluate if a culture environment, whose only source of energy was given by the extract of the fruit of P. aculeata Kunth and Chenopodium ambrosioides L., would allow in vitro- the viability of ciliates to a concentration ($10^4$) similar to the ruminal natural conditions, and make future evaluations about the capability of some plants to eliminate protozoarians.

MATERIALS AND METHODS

This investigation was conducted by the Animal Nutrition and Biotechnology Laboratory of the Faculty of Agriculture Science, Campus IV, UNACH, Chiapas, Mexico.

Culture medium and treatments

For the anaerobic culture environment (AMC, table 1) were added 5 mL of CH$_3$COONa 1.5 %. For the witness treatment (T1), 54 mL of AMC were added in vials of 100 mL with 150 μL of A. sativa L every 9 mL of AMC, 20000 UI of penicillin and 25 mg of streptomycin. For the treatments T2, T3 and T4, AMC was added with the same doses of antibiotics but, instead of A. sativa L; 150, 300 and 450 μL of the soluble extract (weight: volume) of 2.0 g of P. aculeata Kunth in 10 mL of distilled water were incorporated to the system of culture. In the T5 treatment, 300 μL of the soluble extract (weight: volume) of 2.0 g of Ch. ambrosioides L in 10 mL of distilled water were added to the medium.

Table 1. Anaerobic culture medium (AMC) for the maintenance of ruminal ciliate protozoa.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Amount per 100 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water, mL</td>
<td>47.6</td>
</tr>
<tr>
<td>Clarified ruminal fluid (CRF), mL (1)</td>
<td>30.00</td>
</tr>
<tr>
<td>Mineral solution I, mL (2)</td>
<td>5.00</td>
</tr>
<tr>
<td>Mineral solution II, mL (3)</td>
<td>5.00</td>
</tr>
<tr>
<td>Sodium carbonate 8 %, mL (4)</td>
<td>5.00</td>
</tr>
<tr>
<td>Sulfide-cystein solution, mL (5)</td>
<td>2.00</td>
</tr>
<tr>
<td>Rezarsurin 0.1% solution, mL (6)</td>
<td>0.1</td>
</tr>
<tr>
<td>Sodium acetate 1.5 % solution, mL (7)</td>
<td>5.00</td>
</tr>
<tr>
<td>Trypticase-pepton, g</td>
<td>0.20</td>
</tr>
<tr>
<td>Levadure extract, g</td>
<td>0.10</td>
</tr>
</tbody>
</table>

1) Clarified ruminal fluid was filtrated and centrifuged to 17664 g for 15 minutes, and sterilized 20 minutes to 21 °C at 15 psi. (2) In every 1000 mL; 6 g K$_2$HPO$_4$ (3) In every 1000 mL of H$_2$O; 6 g KH$_2$PO$_4$, 6 g (NH$_4$)$_2$SO$_4$, 12 g NaCl, 2.45 g MgSO$_4$ and 1.6 g CaCl$_2$:H$_2$O. (4) 8 g Na$_2$CO$_3$ per 100 mL of distilled H$_2$O. (5) 2.5 g L-cystein (in 15 mL 2N NaOH) + 2.5 g Na$_2$S·9H$_2$O (in 100 mL of H$_2$O). (6) 0.1 mL resazurin in 100 mL suggested by Ley de Coss et al (2011a, 2011b). (7) 5 mL of CH$_3$COONa 1.5 % suggested by Dehority (2008).

Soluble extract obtention

P. aculeata Kunth fruits and Ch. ambrosioides L. forage were collected in the Soconusco area, Chiapas, Mexico. The samples were dehydrated by natural conditions and dried in a stove (VWR International, Sheldon, U.S.A.) at 40°C until 13% of moisture; grinded in a Wilell mill (ED-5, Tomas-Willey Mill, U.S.A.) with 1 mm sieve and stored in amber glass containers. To get the soluble extract, 2.0 g dry matter of each plant suspended in 10 mL of distilled water were deposited in tubes of 18 x 150 mm; then this suspension was mixed 3 minutes in a vortex (Science
MED, MX-S, U.S.A.) and let it repose 5 minutes. The supernatant fluid represents the soluble extract.

**Protozoa concentrate obtention**

The ruminal fluid (FR) was collected through an esophagi cannula of the nearest reticule-rumen of a F1 bovine (zebu x Sweden; 450 kg LW) nourished with *Cynodon plectotachus* K. (Schum) Pilger and 3.0 kg d⁻¹ of a supplement with 13% protein made with maize, sorgum, soybean paste, urea and a mix of: 1) macrominerals (%), Ca (17), (12), Mg (5), Na (7), Cl (10.59), K (0.04) and S (0.05); 2) vitamins (UI), A (350,000), D (150,000), E (150); 3) microminerals (ppm), Mn (4,000), F (2,939), Zn (6,000), Cu (1,000), I (500), Se (40), Co (60). This diet was offered for 7 days before collecting the ruminal fluid which was filtered using a 75 mm-diameter-filtration-conical-funnel (Mod. TS5190N11, Kimax, Mexico) with cotton crapes; and incubated at 38 ± 0.5°C under CO₂ conditions. After that, the protozoa concentrate was taken through a 500 mL-separation-glass-funnel (Mod. K29034f, Kimax, Mexico) for 60 minutes to had the protozoa sedimentation which were dissolved in the cultivate medium without carbohydrate source.

**Inoculation and protozoa calculation**

The treatments were inoculated under aseptic conditions in presence of CO₂ (95 % purity, INFRA®, Mexico) with 6.0 mL of protozoa concentrate which was obtained with the previous described method. The inoculated mediums were incubated (Felisa FE-133A, Feligneo, Mexico) to 38 ± 0.5 °C. When the incubation period ended (3, 6, 12, 24, 48 y 72 h), the inoculated mediums were homogenized and 0.5 mL were taken out with a pipette; the direct protozoa alive count was made with a phase-contrast microscope (40X, Biológico BX51, Olympus, U.S.A.) using the technique and calculation formulas described by Ley de Coss et al. (2011a; 2011b). Moreover, a sample of 1.0 mL of medium was extracted under anaerobic conditions to determine pH with a pH-meter (Orion A250, Orion Research, Inc., U.S.A.); the samples were taken conserving temperature and anaerobic conditions with the purpose of reducing protozoa death. For the VFA analysis, 2.5 mL of cultivate medium were transported at 3, 6, 12, 24, 48 and 72 h of incubation and were centrifuged for 10 min at 17,664 g; 2.0 mL of supernatant fluid were mixed with 1 mL of 25%-metaphosphoric acid; the VFA were determined by gasses-chromatography (Claurus 500, Perkin-Elmer™, U.S.A.) with the conditions described by Ley de Coss et al. (2011a; 2011b). Dry matter *in-vitro* degradability (DMiD) of *P. aculeata* Kunth was calculated with cultivate tubes (18 x 150 mm) where 0.3 ± 0.01 g DM were added and sterilized with an autoclave (Felisa, FE-397, Mexico), then 9.0 mL of cultivate medium were added to each tube under anaerobic conditions (presence of CO₂). The tubes were inoculated with 1 mL of CRF and incubated for 3, 6, 12, 24 and 48 h at 38 ± 0.5 °C; by the end of the incubation period, the amount of DM was estimated.

**Experimental design and statistical analysis**

The experimental design was completely randomized and the data of DMiD, VFA concentration and pH of the cultivate mediums was analyzed with GLM procedure of SAS, while protozoa concentration in the cultivate medium was studied through Kruskal-Wallis proof with independents ranges of Wilcoxon. All the means were compared with Tukey proof (p ≤ 0.05) and the statistical package (13-11) was used in the complete study.

**RESULTS**

**Protozoa concentration and dry matter degradability**

Differences had been found (p≤0.05) in protozoa concentration (Table 2) between treatments; at 3h of incubation, the treatments T3 (300 µL) and T4 (450 µL) observed the largest amounts of alive cells, compared with the control (T1), T2 and T5. At 6 and 12 h post-incubation, the protozoa concentrations were bigger (p≤0.05) in T1 (control) and T2, while at the end of the incubation period; only T3 and T4 had the largest protozoa concentrations (p≤0.05). So, doses of 300 and 400 µL of extract of *P. aculeata* Kunth kept, under *in-vitro* conditions and 72 h, the biggest amounts of protozoa appertaining to any taxonomical order of ruminal protozoa.

The pH in the cultivate mediums were different (p≤0.05) since 12 h of incubation between treatments, the lowest pH kept in T3 until 48 h; however in the treatments whose been the greater protozoa amount, pH was higher phenomenon which involve that pH affected the increase of those microorganisms. By the way, the effect of *Ch. ambrosioides* L (T5), pH in the medium was lower (p≤0.05) affecting the growth of protozoa.

Dry matter *in-vitro* degradability (DMiD) of *P. aculeata* Kunth was of 24.21, 45.87 y 89.76 % at 6, 12 y 24 h of incubation respectively; after 24 h there was no residual material, so apparently was a total degradability.

**Volatile Fatty Acids (VFA) concentration**

The amount of acetate through the first 6 h of incubation did not change (p>0.05) between
treatments (Table 3); although, it was observed the indirect proportion between the amount of *P. aculeata* Kunth extract and the production of acetate, to greater amounts of *P. aculeata* Kunth extract, less acetate production. While the propionic concentration was similar (p > 0.05) between treatments, there was difference at 12 h between the control and treatments with *P. aculeata* Kunth about the butyric acid concentration; although, with the defauning plant was a smaller amount of that VFA for 72 h (p ≤ 0.05), just at the ending of the incubation period. This information shows that *P. aculeata* Kunth extract reduces acetic and butyric concentration but does not modify the amount of propionic. The total concentration of VFA at 72 h was smaller (p ≤ 0.05) in treatments 4 and 5; the last one had the *Ch. ambrosioides* L.

**DISCUSSION**

The concentrations and protozoa species were similar through the entire study; that is why the fruit that had been evaluated has potential as nutrients source, similar at the data reported by Coleman (1981) with the development *in-vitro* of Order Entonidios protozoa in flour-based mediums of *Musa* spp. L. and *Oryza sativa* L.; when those mediums were substituted for *O. sativa* L. starch (4.6 x10³ mL⁻¹) and *T. aestivum* L., observed the development of *Entodonium caudatum* Stein, *Epidinium ecaudatum* F. y *Ophryoscole* caudatus Eberlein while in mediums made with de *Canavalia ensiformis* (L.) DC flour, growth (2.9 x10⁴ ciliates mL⁻¹) *E. caudatum* y *E. ecaudatum caudatum* for 9 months (Williams y Coleman, 1992). According to Teferedegne et al. (1999), the ruminal microorganisms are adaptable to chemical substances with defauning capabilities, which involve the action of ruminal bacteria which at the same time, have repercussions on the FVA production because of the adaptation and metabolism of the chemical secondary substances (Ivan et al., 2003).

The defauning effect of *Ch. ambrosioides* L. (T5) was reported by Ley de Coss et al (2011B); also mentioned 2.0 x10⁴ protozoa mL⁻¹ of medium when *A. sativa* L. and some tuber starch were used (Dehority, 2008; Ley de Coss et al., 2011a). In this investigation, protozoa of all the taxonomic orders reported in the rumen were observed while Dehority, 2008 mentioned a medium where only growth *Epidinium cadatum* y *Entodonium caudatum*. All this information is important because one of the elementary factors for protozoa *in-vitro* cultivate is the change between saturated medium and a fresh one; besides from addition antibiotics to regulate growth and bacterial population; this allows realize studies about nutritional requirements and metabolic activity of this microorganisms (Onodera y Henderson, 1980; Martin et al., 1999; Dehority, 2008).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incubation period (h)</th>
<th>3</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1, Control</td>
<td></td>
<td>3.00b</td>
<td>4.40a</td>
<td>4.23ab</td>
<td>2.50c</td>
<td>2.00a</td>
<td>1.17b</td>
</tr>
<tr>
<td>T2, 150 µL‡</td>
<td></td>
<td>3.00b</td>
<td>3.53d</td>
<td>4.27a</td>
<td>2.17c</td>
<td>1.93a</td>
<td>1.20b</td>
</tr>
<tr>
<td>T3, 300 µL‡</td>
<td></td>
<td>3.40a</td>
<td>4.23c</td>
<td>3.87bc</td>
<td>3.90a</td>
<td>1.27bc</td>
<td>1.96a</td>
</tr>
<tr>
<td>T4, 450 µL‡</td>
<td></td>
<td>3.83a</td>
<td>4.30b</td>
<td>3.30cd</td>
<td>2.77b</td>
<td>1.96ab</td>
<td>2.30a</td>
</tr>
<tr>
<td>T5, 300 µL§</td>
<td></td>
<td>2.87c</td>
<td>1.00e</td>
<td>0.67d</td>
<td>0.03d</td>
<td>0.02c</td>
<td>0.00d</td>
</tr>
<tr>
<td>SEM€</td>
<td></td>
<td>1.33</td>
<td>1.00</td>
<td>1.30</td>
<td>1.08</td>
<td>2.16</td>
<td>1.48</td>
</tr>
</tbody>
</table>

Means with different letter in the same column are different (p ≤ 0.05); € Standard Error; ‡Solution made with 2 g *P. aculeata* Kunth in 10 mL of water; § Solution made with 2 g *Ch. ambrosioides* L. in 10 mL of water.
Different nutrients sources (energy, protein and mineral) had been used in \textit{in-vitro} cultivation technics of ruminal ciliate protozoa (Ivan y Entz, 2004; Dehority, 2008). In the present research the cultivate was obtained when the fruit of \textit{P. aculeata} Kunth, \textit{A. sativa} L. and a mineral mix were added (Table 1); in other reports, nutritional broths based in \textit{Triticum aestivum} L. 1.5\% \textit{Dactylis glomerata} L. 1\%, solutions of \textit{T. aestivum} L. 1.5\% plus \textit{Medicago sativa} L. 1\%, \textit{Sorghum vulgare}, \textit{Sorghum bicolor} (L.) Moench 5\% plus \textit{Panicum maximum} Jacq. 5\% (Dehority, 1998; 2008) had been used. The antibiotics doses that were added to the cultivate medium allowed the development of Holotricha class protozoa (10^7 cells mL^{-1}), while Lee \textit{et al.} (2000) mention that a dose of 0.1 mg mL^{-1} of streptomycin sulfate, penicillin and cloramphenicol keeps stable pH to 6.53, but the metabolic activity of ciliate protozoa was inhibited for the presence of fungi and bacteria in cultivate mediums.

\begin{table}[h]
\centering
\caption{Volatile Fatty Acids (VFA) concentration in cultivate mediums with \textit{P. aculeata} Kunth y \textit{Ch. ambrosioides} L.}
\begin{tabular}{lcccccccc}
\hline
Time, hours & 0 & 3 & 6 & 12 & 24 & 48 & 72 \\
\hline
\textbf{Acetic acid, mM L^{-1}} & & & & & & & \\
T1, control & 22.90a & 23.75a & 23.93ab & 25.34a & 34.36ab & 31.63b & 42.25ab \\
T2, 150 µL‡ & 22.51a & 24.95a & 23.91ab & 23.78a & 40.18a & 38.85ab & 46.42ab \\
T3, 300 µL‡ & 23.96a & 26.08a & 25.04a & 21.34a & 42.31a & 42.61b & 55.77a \\
T4, 450 µL ‡ & 18.25a & 23.80a & 22.70b & 23.12a & 25.33b & 22.77c & 25.30b \\
T5, 300 µL§ & 20.23a & 19.89a & 18.67c & 16.78c & 16.22c & 15.55c & 19.23b \\
SEM§ & 4.08 & 1.06 & 0.73 & 2.98 & 4.45 & 3.97 & 8.23 \\
\hline
\textbf{Propionic acid, mM L^{-1}} & & & & & & & \\
T1, control & 4.50a & 4.71a & 4.79a & 4.93a & 4.53a & 4.21a & 4.71a \\
T2, 150 µL‡ & 4.41a & 4.86a & 4.67a & 4.14a & 4.04a & 4.09a & 3.03a \\
T3, 300 µL‡ & 4.59a & 5.04a & 4.74a & 3.81a & 3.97a & 3.40a & 3.33a \\
T4, 450 µL ‡ & 3.77a & 4.91a & 4.69a & 4.78a & 4.41a & 4.25a & 3.28a \\
T5, 300 µL§ & 4.00a & 4.56a & 4.56a & 4.54a & 4.23a & 4.65a & 3.89a \\
SEM§ & 0.85 & 0.20 & 0.14 & 0.52 & 0.26 & 0.41 & 0.90 \\
\hline
\textbf{Butyric acid, mM L^{-1}} & & & & & & & \\
T1, control & 1.87a & 1.97a & 1.97a & 2.02a & 2.03a & 3.63a & 4.96a \\
T2, 150 µL‡ & 1.75a & 2.02a & 1.90a & 1.65a & 1.79ab & 2.68ab & 3.81ab \\
T3, 300 µL‡ & 1.78a & 2.06a & 2.01a & 1.55a & 1.67b & 1.71bc & 3.49ab \\
T4, 450 µL ‡ & 1.59a & 2.05a & 1.97a & 2.04a & 1.84ab & 1.27c & 1.68b \\
T5, 300 µL§ & 1.76a & 2.34a & 1.00b & 1.02b & 1.34b & 1.12c & 0.78c \\
SEM§ & 0.37 & 0.09 & 0.12 & 0.21 & 0.33 & 0.41 & 1.23 \\
\hline
\textbf{Total VFA, mM L^{-1}} & & & & & & & \\
T1, control & 29.27a & 30.43a & 30.71a & 32.3a & 40.93ab & 40.14ab & 56.26a \\
T2, 150 µL‡ & 28.67a & 31.48a & 30.48a & 29.41a & 46.01a & 49.29a & 52.59a \\
T3, 300 µL‡ & 33.33a & 33.19a & 31.80a & 26.71a & 47.95a & 47.72a & 67.26a \\
T4, 450 µL ‡ & 23.62a & 30.76a & 29.36b & 29.94a & 31.59b & 31.63b & 30.26b \\
T5, 300 µL§ & 25.99a & 26.79a & 24.23c & 22.34b & 21.79c & 21.32c & 23.9b \\
SEM§ & 5.29 & 1.35 & 0.90 & 3.76 & 4.44 & 4.62 & 8.68 \\
\hline
\end{tabular}
\footnotesize{a,b,c} Means with different letter in the same column are different (p ≤ 0.05); \footnotesize{‡} Standard Error \footnotesize{§}Solution made with 2 g \textit{P. aculeata} Kunth in 10 mL of water; \footnotesize{§} Solution made with 2 g \textit{Ch. ambrosioides} L. in 10 mL of water.}
\end{table}
About DMiD of Parmentiera aculeata Kunth fruit, rates from 87.8 to 100% of degradability has been reported (Moctezuma et al., 1993); in the other hand García-Castillo et al. (2008) mention that in-situ degradability of Parmentiera aculeata Kunth fruit was about 33.3% at the first 24 h of introduced the samples in the rumen; although there is a significant difference between results because of the ripeness conditions of the fruit utilized: in this study, a mature fruit was used; while García-Castillo et al. (2008) used a green P. aculeata Kunth fruit, that is why the ripeness affected the dry matter degradability including the ruminal protozoa growth. The amounts obtained in this study can be related to the solubility of the dry-grinded fruit and the high content of organic matter (OM), especially soluble fiber. In relation to, García-Castillo et al. (2008) mention that P. aculeata Kunth fruit contents 14.7% DM, 91.2% OM, 2.8% CP and 82.5% fiber; about this results, Sosa et al. (2004) indicate that there is not a direct relation between the variation in the NDF content and DMiD in forage plants; just the lignification rate could reduce the degradability of the dry matter.

When the amount of P. aculeata Kunth fruit extract increased in the medium, the production of acetic acid reduced, about this Ley de Coss et al., (2011a) shown that defaunating plants like Ch. ambrosioides L. reduces the acetic concentration in the medium, similar results were reported by Nagaraja et al. (1992) and Mendoza et al. (1993); but this result does not coincide with Ivan et al. (2004) whose study indicates that Enterolobium cyclocarpum (Jacq.) Griseb, defaunating plant does not affect VFA concentration but reduces the amount of ciliate protozoa like a transitory effect. Abreu et al. (2004) indicate a smaller amount of acetic and larger of propionic acid in oives nourished with Sapindus saponaria L. fruit, besides increase 67% protozoa concentration with a rate of 121 mM L⁻¹ total VFA which are superior at the rate found in this study. On that score, Ivan et al. (2000) show the FVA concentration depends of the ciliate protozoa specie and if Dasytricha predominate, the amount of total VFA would be 73.5 mM L⁻¹ and 88.1 mM L⁻¹ if the predominant specie would be Isotricha in diets based in corn forage.

In tropical regions, in important to analyze nutritional alternatives for the balance of glucogenics and acetogenics fatty acids in animals because 90% of glucose comes from neogenesis, specially from propionic acid, and 70% of the energy comes from the VFA that are produced in the rumen (Dehory, 2003), using fruits provided of forage plants has allowed increase the amount of propionic, improving the balance of gluconeogenic fats acids according to Navas-Camacho et al. (1994).

CONCLUSION

In conclusion, the hydro-soluble fraction of P. aculeata Kunth fruit allows to keep an approximate amount of 2.3 x10² protozoa per mL of medium without alterations on the pH range when the extract dose is superior to 300 µL. Only the largest extract applied dose reduced the total VFA concentration, particularly acetic and butyric acids without changes in propionic. The DMiD was high from 24 h of incubation, which allowed disposing nutrients for the microorganisms. The medium would allow the evaluation of defaunating capability of forage plants which secondary compounds

Acknowledgments

To National Council of Science and Technology (CONACYT) for the investment of this investigation as a part of the project named “Estimation and ambient impact of carbon capture in Elaeis guineensis Jacq plantations in the State of Chiapas, Mexico” which is part of the Scientific Development to attend National Problems Project (PDCPN2013-01) and the scholarship of Willians de León de León (Scholarship holder 284222) to make his studies in the program of Master in Tropical Farming Production Sciences at the Autonomous University of Chiapas.

REFERENCES


