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]

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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 ($p\leq0.05$) 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 largest amounts ($p\leq0.05$) compared with the other treatments, especially with T5. There was no difference ($p\leq0.05$) 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 ($p\leq0.05$) 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 Degradación *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 ($p\leq0.05$), 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 ($p\leq0.05$) con respecto al resto de los tratamientos, principalmente con T5. Hubo diferencia ($p\leq0.05$) en la concentración de ácido acético entre T1, T2 y T3 con respecto a T4 a las 72 h y con T5 desde las 6 h de incubación, además, para ácido butírico, el T5 tuvo la menor concentración ($p\leq0.05$) 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-nutritionals 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 10^5 to 10^6 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 (Micha£owski *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_2HPO_4, KH_2PO_4, $(NH_4)_2SO_4$, NaCl, $MgSO_4$, $CaCl_2{\cdot}H_2O$, Na_2CO_3 8%, L–cystein (dissolved in 2N NaOH) + Na_2S-$9H_2O$ with oxide-reduction indicators like resazurin and CH_3COONa 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_3COONa 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 distillated 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 distillated water were added to the medium.

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

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

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_2HPO_4. (3) In every 1000 mL of H_2O; 6 g KH_2PO_4, 6 g $(NH_4)_2SO_4$, 12 g NaCl, 2.45 g $MgSO_4$ and 1.6 g $CaCl_2{\cdot}H_2O$. (4) 8 g Na_2CO_3 per 100 mL of distillated H_2O. (5) 2.5 g L–cystein (in 15 mL 2N NaOH) + 2.5 g Na_2S-$9H_2O$ (in 100 mL of H_2O). (6) 0.1 mL resazurin in 100 mL suggested by Ley de Coss *et al* (2011a, 2011b). (7) 5 mL of CH_3COONa 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 Willey 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 distillated 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 plectotaschyus* 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 CO_2 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 CO_2 (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 ºC. 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 determinate 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 retired 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 determinated 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 fruits 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 CO_2). 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 ($p \leq 0.05$) and the statistical package (13-11) was used in the complete study.

RESULTS

Protozoa concentration and dry matter degradability

Differences had been found ($p \leq 0.05$) 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 ($p \leq 0.05$) in T1 (control) and T2, while at the end of the incubation period; only T3 and T4 had the largest protozoa concentrations ($p \leq 0.05$). 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 ($p \leq 0.05$) 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 ($p \leq 0.05$) 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 ($p \leq 0.05$), 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 ($p \leq 0.05$) 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 x10^3 mL^{-1}) and *T. aestivum* L., observed the development of *Entodonium caudatum* Stein, *Epidinium ecaudatum* F. y *Ophryoscolex caudatus* Eberlein while in mediums made with de *Canavalia ensiformis* (L.) DC flour, growth (2.9 x10^3 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 x10^4 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 2. Protozoa concentration (10^4) in cultivate mediums with *P. aculeata* Kunth y *Chenopodium ambrosioides* L.

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

a, b, c, d Means with different letter in the same column are differents ($p \leq 0.05$); € 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.

Table 3. Volatile Fatty Acids (VFA) concentration in cultivate mediums with *P. aculeata* Kunth y *Ch. ambrosioides* L.

Time, hours	0	3	6	12	24	48	72
	Acetic acid, mM L^{-1}						
T1, control	22.90^{a}	23.75^{a}	23.93^{ab}	25.34^{a}	34.36^{ab}	31.63^{b}	42.25^{ab}
T2, 150 µL‡	22.51^{a}	24.95^{a}	23.91^{ab}	23.78^{a}	40.18^{a}	38.85^{ab}	46.42^{ab}
T3, 300 µL‡	23.96^{a}	26.08^{a}	25.04^{a}	21.34^{a}	42.31^{a}	42.61^{a}	55.77^{a}
T4, 450 µL ‡	18.25^{a}	23.80^{a}	22.70^{b}	23.12^{a}	25.33^{b}	22.77^{c}	25.30^{b}
T5, 300 µL§	20.23^{a}	19.89^{a}	18.67^{c}	16.78^{c}	16.22^{c}	15.55^{c}	19.23^{b}
SEM€	4.08	1.06	0.73	2.98	4.45	3.97	8.23
	Propionic acid, mM L^{-1}						
T1, control	4.50^{a}	4.71^{a}	4.79^{a}	4.93^{a}	4.53^{a}	4.21^{a}	4.71^{a}
T2, 150 µL‡	4.41^{a}	4.86^{a}	4.67^{a}	4.14^{a}	4.04^{a}	4.09^{a}	3.03^{a}
T3, 300 µL‡	4.59^{a}	5.04^{a}	4.74^{a}	3.81^{a}	3.97^{a}	3.40^{a}	3.33^{a}
T4, 450 µL ‡	3.77^{a}	4.91^{a}	4.69^{a}	4.78^{a}	4.41^{a}	4.25^{a}	3.28^{a}
T5, 300 µL§	4.00^{a}	4.56^{a}	4.56^{a}	4.54^{a}	4.23^{a}	4.65^{a}	3.89^{a}
SEM€	0.85	0.20	0.14	0.52	0.26	0.41	0.90
	Butyric acid, mM L^{-1}						
T1, control	1.87^{a}	1.97^{a}	1.97^{a}	2.02^{a}	2.03^{a}	3.63^{a}	4.96^{a}
T2, 150 µL‡	1.75^{a}	2.02^{a}	1.90^{a}	1.65^{a}	1.79^{ab}	2.68^{ab}	3.81^{ab}
T3, 300 µL‡	1.78^{a}	2.06^{a}	2.01^{a}	1.55^{a}	1.67^{b}	1.71^{bc}	3.49^{ab}
T4, 450 µL ‡	1.59^{a}	2.05^{a}	1.97^{a}	2.04^{a}	1.84^{ab}	1.27^{c}	1.68^{b}
T5, 300 µL§	1.76^{a}	2.34^{a}	1.00^{b}	1.02^{b}	1.34^{b}	1.12^{c}	0.78^{c}
SEM €	0.37	0.09	0.12	0.21	0.33	0.41	1.23
	Total VFA, mM L^{-1}						
T1, control	29.27^{a}	30.43^{a}	30.71^{a}	32.3^{a}	40.93^{ab}	40.14^{ab}	56.26^{a}
T2, 150 µL‡	28.67^{a}	31.48^{a}	30.48^{a}	29.41^{a}	46.01^{a}	49.29^{a}	52.59^{a}
T3, 300 µL‡	33.33^{a}	33.19^{a}	31.80^{a}	26.71^{a}	47.95^{a}	47.72^{a}	67.26^{a}
T4, 450 µL ‡	23.62^{a}	30.76^{a}	29.36^{b}	29.94^{a}	31.59^{b}	31.63^{b}	30.26^{b}
T5, 300 µL§	25.99^{a}	26.79^{a}	24.23^{c}	22.34^{b}	21.79^{c}	21.32^{c}	23.9^{b}
SEM €	5.29	1.35	0.90	3.76	4.44	4.62	8.68

a, b, c, Means with different letter in the same column are differents ($p \leq 0.05$); € 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 *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 *P. aculeata* Kunth, *A. sativa* L. and a mineral mix were added (Table 1); in other reports, nutritional broths based in *Triticum aestivum* L. 1.5%, *Dactylis glomerata* L. 1%, solutions of *T. aestivum* L. 1.5% plus *Medicago sativa* L. 1 %, *Sorghum vulgare*, *Sorghum bicolor* (L.) Moench 5% plus *Panicum maximun* Jacq. 5% (Dehority, 1998; 2008) had been used. The antibiotics doses that were added to the cultivate medium allowed the development of Holotrica class protozoa (10^4 cells mL^{-1}), while Lee *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.

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 DM*iv*D 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 defauning plants like *Ch. ambrosioides* L. reduces the acetic concentration in the medium, similar results were reported by Nagaraja *et al.* (1992) y Mendoza *et al*. (1993); but this result does not coincide with Ivan *et al.* (2004) whose study indicates that *Enterolobium cyclocarpum* (Jacq.) Griseb, defauning 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 ovines 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 (Dehority, 2003), using fruits provided of forage plants has allowed increase the amount of propionic, improving the balance of gluconeogenics fatty 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 $x10^4$ 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 DM*iv*D 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

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