

Ensiling natural meadow forage in «Tierra del Fuego», Argentina

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Abstract

Livestock activity in the Argentinian «Tierra del Fuego» island is based on highly variable natural grasslands (NG). The conservation of fodder, despite the unfavorable environment, is restricted to hay; conservation as silage could be constrained by fodder quality and low temperatures. The objective was to assess the quality of NG silages fermented under contrasting storing conditions. Typical meadow forage was ensiled in minisilos and stored under shelter (Shelter) or in the field (Field) in a complete block design with repeated measures in time (t). Forage was chopped and inoculated with lactic acid bacteria plus enzymes, and harvested with 420 g dry matter (DM) kg⁻¹ fresh matter, 111 g crude protein kg⁻¹ DM, 665 g ash-free neutral detergent fibre kg⁻¹ DM, and 64 g water soluble carbohydrates kg⁻¹ DM. Shelter minisilos had higher metabolizable energy concentration at 236 d ($P_{T_{mat}}$ = 0.03; Shelter = 10.2 ; Field = 9.6 MJ kg⁻¹ DM, $P = 0.01$), and lower DM losses (Shelter = -0.2; Campo = 22%, $P = 0.02$) and values of pH and N-NH₃/total N (Field: 5.6 and 12% and Shelter: 4.4 and 6.8%, $P \leq 0.01$). Fermentation acids concentration was similar for both treatments with preponderance of lactic acid, but acetic acid concentration increased over time ($P_t \leq 0.01$). It was concluded that in «Tierra del Fuego», natural meadow forage quality is compatible with ensiling, but environmental conditions can limit the fermentation process.

Additional key words: forage conservation, natural grasslands, Patagonia, silage.

Resumen

Ensilaje de forraje de vegas naturales en Tierra del Fuego, Argentina

La ganadería en la isla de «Tierra del Fuego» argentina esta basada en pastizales naturales (PN) altamente variables. La conservación de forrajes, pese al ambiente poco propicio, se reduce al heno; la conservación como silaje podría verse limitada por la calidad de los forrajes y las bajas temperaturas. El objetivo fue evaluar la calidad del ensilado de PN fermentado en condiciones de almacenamiento contrastantes. El forraje de una vega típica fue ensilado en minisilos y almacenado bajo protección (Prot) o en el campo (Campo), en un diseño de bloques completos con medidas repetidas en el tiempo (t). El forraje fue picado e inoculado con bacterias ácido-lácticas más enzimas y cosechado con 420 g de materia seca (MS) kg⁻¹ materia fresca, 111 g proteína cruda kg⁻¹ MS, 665 g fibra detergente neutro libre de ceniza kg⁻¹ MS, y 64 g de carbohidratos solubles en agua kg⁻¹ MS. Los minisilos Prot tuvieron mayor concentración de energía metabolizable a los 236 d ($P_{T_{mat}}$ = 0,03; Prot = 10,2, Campo = 9,6 MJ kg⁻¹ MS, $P = 0,01$), y menores pérdidas de MS (Prot = -0,2, Campo = 22%, $P = 0,02$) y valores de pH y N-NH₃/N-total (respectivamente, Prot: 4,4 y 6,8%, Campo: 5,6 y 12%, $P \leq 0,01$). La concentración de ácidos de fermentación fue similar para ambos tratamientos, preponderando el ácido láctico, pero la concentración de ácido acético aumentó con el tiempo ($P_t \leq 0,01$). Se concluyó que en «Tierra del Fuego» la calidad de forraje de PN es compatible con el proceso de ensilaje y que las condiciones ambientales pueden limitar el proceso de ensilaje.

Palabras clave adicionales: conservación de forrajes, Patagonia, praderas naturales, silaje.

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Abbreviations used: ADF_{OM} (ash free insoluble in acid detergent fiber), ADL_{SA} (acid detergent lignin with sulphuric acid), aND-F_{OM} (ash free insoluble in neutral detergent fibre, with α -amylase), Cel (cellulose), CP (crude protein), DM (dry matter), GP (gas production), Hemi (hemicellulose), ivDMD (*in vitro* DM digestibility), LAB (lactic acid bacteria), SEM (standard error of the mean), TN (total nitrogen), VFA (volatile fatty acids), WSC (water soluble carbohydrates).

Introduction

Livestock activity in Tierra del Fuego island (*c.a.* 55° S and 65° W) is characterized, both in Argentine and Chilean sides, by extensive exploitation of sheep and beef cattle grazing natural steppes and meadows in an extreme agroecological environment with severe thermal, water and soil constraints (Jacob *et al.*, 1999). There is a marked seasonality in forage production from late spring to summer, and a winter period with nil productivity, where much of the forage remains on the ground rotting under water, ice and snow. The geographical isolation limits the income of inputs, determining that livestock systems are highly dependent on the direct use of their own forage resources (natural or implanted).

Forage conservation is considered a priority tool to improve Argentine and Chilean livestock production systems in Patagonian region (Livraghi, 1996; Nilo Cavacevich, 2006). Widespread adoption of forage conservation technology would assist in avoiding massive waste of forage accumulated during the growing season, thereby reducing the recurrent forage supply imbalance. Concurrently, it would improve the nutritional status of animals and reduce the costs of winter food.

Although quantitatively limited, hay conserved as big round bales is the main form of preserving forage. However, the high soil moisture and environmental humidity, plus the typical strong winds undermine the efficiency of hay making. In contrast, silage minimizes the exposure of forage in the field, so that it could be more appropriate for the island conditions (Balocchi, 1999). However, to our knowledge, there have not been local experiences in preserving forage as silage. Conservation as silage requires soluble carbohydrates, low buffer capacity, presence of sufficient amount of lactic acid bacteria (LAB) and anaerobic conditions (McDonald *et al.*, 1991). Lactic acid bacteria requires between 5°C and 50°C to develop (Woolford, 1984), but in «Tierra del Fuego» daily temperatures during the harvesting season (January to April) fluctuate around 10°C, close to the minimum values required by LAB to grow. The aim of this work was to assess the quality of natural meadow forage silages fermented under contrasting storing conditions.

Material and methods

Experimental design

Six *c.a.* 100-kg minisilos were made with forage harvested from a typical meadow and were stored

under shelter (Shelter) or directly in the field out-doors (Field) according to a complete block design (blocking by the time elapsed since cutting).

Description of meadow and ensiling technique

The forage was harvested from a fraction of a natural meadow located at the Misión Salesiana of Rio Grande («Tierra del Fuego», 53° 42' 47.9" S, 67° 49' 57.4" W and 7 m asl) at the 25th January 2008 (south hemisphere summer). Aboveground biomass was estimated by cutting 10 random samples with scissors and using a rectangular frame of 0.2 m² (0.2 × 1 m).

Forage was cut and chopped directly with a grass harvester for silage (Mainero, Bell Ville Córdoba-Argentina) and inoculated manually (as indicated by the manufacturer: 4 L solution/ton of forage with 250 g inoculum/100 L water). The inoculant contained a mixture of enzymes (amylase and cellulase) and LAB (*Lactobacillus plantarum*, *Streptococcus faecium*, *Pediococcus acidilactici*; Sill-All, Alltech Biotechnology SRL, Pilar, Buenos Aires). The forage was carefully compacted into an appropriate section of silage storage bags (IPESA, Rio Chico, Rio Grande, Tierra del Fuego) and set in wooden minisilos *c.a.* 1 m³. Bags were subsequently sealed with tape, and minisilos were moved to their definite place of storage (Field or Shelter) and pressed with bags filled with gravel (*c.a.* 70 kg m⁻²). Minisilos in Shelter treatment were kept in a big barn where machinery was sheltered, and no animal or source of heat was available.

Observations and sampling

Daily minimum and maximum temperatures from «Rio Grande» («Tierra del Fuego») were obtained from the National Meteorological Service. Temperature within the minisilos was monitored on days 1, 3, 111 and 236 (t_0 , t_1 , t_2 , t_3 corresponding to 25th and 28th January, 15th May, and 17th September 2008 respectively), using a digital thermometer (Maxthermo MD 3003 type K; Tainan, Taiwan) with a Inconel 600 probe of 0.6 m length, and reported as the temperature difference with respect to the environment temperature (recorded with the same instrument).

During harvesting, forage samples were taken, and then each minisilo was sampled at 111, 236 and 447 (t_4 , 16th April 2009) days with a wool core sampler. All

samples were preserved in plastic bags at -18°C until processed in the laboratory.

Minisilos were weighed on days 0 and 447 using an electronic balance (EziWeit 2; Tru-Test Co., Auckland, New Zealand).

Analytical methods

All procedures were adjusted to the standardized protocols proposed by the PROMEFA (Jaurena and Wawrzkiwicz, 2008). In short, samples were characterized by dry matter (DM) and ash (AOAC, 1984) content. Crude protein (CP = total N \times 6.25) was determined by Kjeldahl (AOAC, 1984) with a Pro-Nitro[®] (Selecta J.P., Barcelona, Spain). Neutral detergent fiber with α -amylase (aNDF_{OM}), acid detergent fiber (ADF_{OM}), and acid detergent lignin with sulphuric acid (ADL_{SA}) were reported ash-free (Van Soest *et al.*, 1991), and determined with an ANKOM[®] equipment (220 model) according to manufacturer recommendations (ANKOM, 2010a,b). Hemicellulose (Hemi) was estimated by difference between aNDF_{OM} and ADF_{OM}, and cellulose (Cel) as the difference between ADF_{OM} and ADL_{SA}. Water soluble carbohydrates (WSC) were determined by the Antrona method (Yemm and Willis, 1954) and silage samples were characterized by pH (Playne and McDonald, 1966) and N-NH₃ (Bremner and Breitenbeck, 1983) content at 3, 111 and 236 days (28th January, 15th May and 17th September 2008, respectively).

Volatile fatty acids (VFA, *i.e.* acetic, propionic, butyric and valeric acids) were measured on purified samples treated with orthophosphoric acid (2% v/v) at a rate of 0.2 mL per each 0.8 mL sample and later centrifuged for 10 min ($\times 10,000$ g). Determination of VFA was made by gas chromatography with a Konik-3000 equipment and a BP-20 column (Mark SGE) using N₂ as carrier and according to protocol recommended by the column manufacturer (adaptation, SUPELCO. «Analyzing fatty acid by packed column gas chromatography. G C. Bulletin 856 A»; Friggens *et al.*, 1998). Lactic acid was measured calorimetrically according to Barnet (1951).

The metabolizable energy content (Mcal kg⁻¹ DM) was estimated with the summative formula of Van Soest (Goering and Van Soest, 1970), and *in vitro* DM digestibility (ivDMD) was determined by the technique of Goering and Van Soest (1970). Simultaneously, in the same *in vitro* system, kinetics of organic matter digestion was described by the *in vitro* gas production

technique proposed by Brooks and Theodorou (1997) and adapted by Wawrzkiwicz and Danelón (2004).

Statistical procedures

All variables, except for ivDMD, were analyzed according to a complete block design (blocking by the order in which minisilos were filled up) and using a model of repeated measures (t: date sampling) where treatment, block and minisilo were considered «fixed» factors (Proc mixed, SAS Institute, 2002).

Dry matter losses were estimated by mass difference between making (t₁, 25th January 2008) and opening time (t₄, 16th April 2009, *i.e.* 447 days) and ivDMD (t₃, 16th September 2008) were analyzed by a non parametric test [«Signed rank tests» (Pappas and DePuy, 2009)] according to a paired sample design.

Incremental rate of gas production (GP) at 6 h, 12 h (cumulative GP at 12 h - GP at 6 h), 24 h (GP at 24 h - GP at 12 h), 48 h (GP at 48 h - GP at 24 h) and 72 h (GP at 72 h - GP at 48 h) was analyzed by ANOVA according to a complete block design.

Results

Aboveground forage biomass yielded 11,245 kg DM ha⁻¹ (SD = 3,618 kg ha⁻¹). The meadow was dominated by *Alopecurus magallanicus* and *Hordeum pubiflorum*, with a quantitatively lower presence of *Carex sp.*, *Poa pratensis* and *Deschampsia flexuosa*. The dominant species at harvest (25th January 2008) were in flowering state.

The forage had the following chemical composition (mean \pm SEM): 420 \pm 15.5 g DM kg⁻¹ fresh matter, 75 \pm 1.3 g ash kg⁻¹ DM, 111 \pm 4.2 g CP kg⁻¹ DM, 665 \pm 3.3 g aNDF_{OM} kg⁻¹ DM, 327 \pm 2.9 g ADF_{OM} kg⁻¹ DM, 26 \pm 1.5 g ADL_{SA} kg⁻¹ DM, 338 \pm 1.7 g Hemi kg⁻¹ DM, 301 \pm 3.0 g Cel kg⁻¹ DM and 64 \pm 3.0 g WSC kg⁻¹ DM.

Minimum and maximum weekly mean temperatures are presented in Figure 1. Difference between minisilos and the outside temperature fell quickly from *c.a.* 2°C to 0°C during the first three days after sealed. Minisilos kept under Shelter had better color (green-yellowish) and odor (acidic) than those stored outdoors, which also had mycelium development. These differences were coincident with DM losses, as indoor minisilos had losses (no different from zero, $P > 0.05$), which were significantly lower than those obtained from units kept outdoors (-0.2 and 22 % respectively, $P = 0.02$).

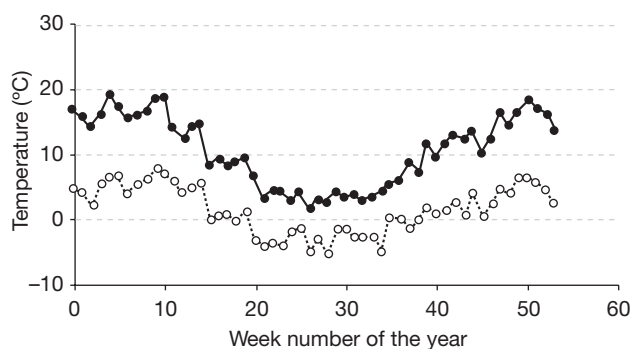


Figure 1. Weekly minimum and maximum mean temperatures for Rio Grande meteorological station (Argentina) during 2008.

Except for ADF_{OM} , Cel, lactic acid and VFA ($P > 0.05$), most variables had differences between forage ensiled under Shelter or in the Field (Table 1). Original forage CP content was $111 \text{ g kg}^{-1} \text{ DM}$ ($SEM = 4.2$), and it increased slightly throughout the fermentation process ($P = 0.03$).

Contents of ADL_{SA} and ash increased over the process of silage. Direct ash mean content rose from $82 \text{ g kg}^{-1} \text{ DM}$ (t_1) up to 94 and $86 \text{ g kg}^{-1} \text{ DM}$ at 236 (t_3) d of fermentation, respectively for Field and Shelter storing systems ($P_t = 0.0003$). Similarly, direct ADL_{SA} mean increased from $28 \text{ g kg}^{-1} \text{ MS}$ (t_1) to 36 and 31 g kg^{-1}

DM respectively for Field and Shelter at t_3 . Furthermore, silage from Shelter treatment had lower $aNDF_{OM}$ content ($P = 0.06$) than Field ones, and lower Hemi content ($P = 0.02$), appearing dramatically reduced with respect to the Field treatment by the last sampling time ($P_{Treat \times t} = 0.02$; 337 and $301 \text{ g Hemi kg}^{-1} \text{ DM}$ at t_3 for Field and Shelter respectively). However, $aNDF_{OM}/ADL_{SA}$ and Hemi/ ADL_{SA} ratios indicated that there only existed a time effect ($P_t \leq 0.02$; $aNDF_{OM}/ADL_{SA}$ ratio: 23b, 25a and 20c g g^{-1} ; Hemi/ ADL_{SA} ratio: 11b, 12a and 10c g g^{-1} ; for t_1 , t_2 and t_3 respectively. Numbers followed by different letters differ, $P < 0.05$) without noticeable differences between treatments ($P > 0.05$).

Though, $ivDMD$ did not reach statistical significance between treatments, at t_3 ($P_{Treat \times t} = 0.03$), the estimated metabolizable energy concentration of forage from Shelter treatment exceeded that from outdoors' minisilos (10.2 and $9.6 \text{ MJ kg}^{-1} \text{ DM}$, respectively, $EE = 0.12$, $P = 0.01$).

Silage samples from minisilos kept indoors yielded 9% ($P > 0.05$) more cumulative gas production than those stored in the Field (Fig. 2), but the greatest difference was brought about by the gas produced during the first 6 h of incubation (Field = 23, Shelter = $50 \text{ mL kg}^{-1} \text{ DM}$, $P = 0.06$), as can be clearly appreciated by the hourly

Table 1. Chemical composition of natural meadow forage stored in the Field or under Shelter. Adjusted means, expressed in $\text{g kg}^{-1} \text{ DM}$, unless stated otherwise

Variable	Treatments		SEM ¹	Factors and significance ²			
	Field	Shelter		Treat	t	Treat × t	Block
<i>Chemical composition</i>							
Dry matter (DM, g kg^{-1} fresh matter)	328	340	8.1	0.33	0.19	0.03	0.03
Ash	87	85	5.2	0.05	<0.01	0.02	0.03
Crude protein	111	114	2.1	0.29	0.03	0.65	0.53
$aNDF_{OM}$	672	647	8.2	0.06	0.49	0.14	0.44
ADF_{OM}	344	333	4.9	0.16	0.36	0.42	0.45
Lignin	30	29	0.7	0.22	<0.01	0.04	0.11
Hemicellulose	328	314	3.5	0.02	0.57	0.03	0.19
Cellulose	314	304	4.5	0.18	0.21	0.57	0.57
<i>In vitro</i> DM digestibility	681	707	11.1	0.18	NA ³	NA	0.24
Metabolisable energy ($\text{MJ kg}^{-1} \text{ DM}$)	10.3	10.5	0.08	0.25	<0.01	0.03	0.14
<i>Fermentative profile</i>							
pH	5.6	4.4	0.17	<0.01	<0.01	<0.01	0.10
$\text{N-NH}_4/\text{TN}^4$ (%)	12.0	6.8	0.91	<0.01	<0.01	<0.01	0.07
Lactic acid	56	59	3.6	0.52	0.28	0.82	0.22
Acetic acid	8	12	1.0	0.02	<0.01	0.05	0.84
Total fermentation acids	64	71	4.2	0.24	0.24	0.97	0.28
Lactic:acetic ratio	9.7	8.7	0.84	0.39	<0.01	0.78	0.85

¹ Standard error of the mean. ² Treat: treatment; t: time. ³ NA: not available. ⁴ TN: total nitrogen.

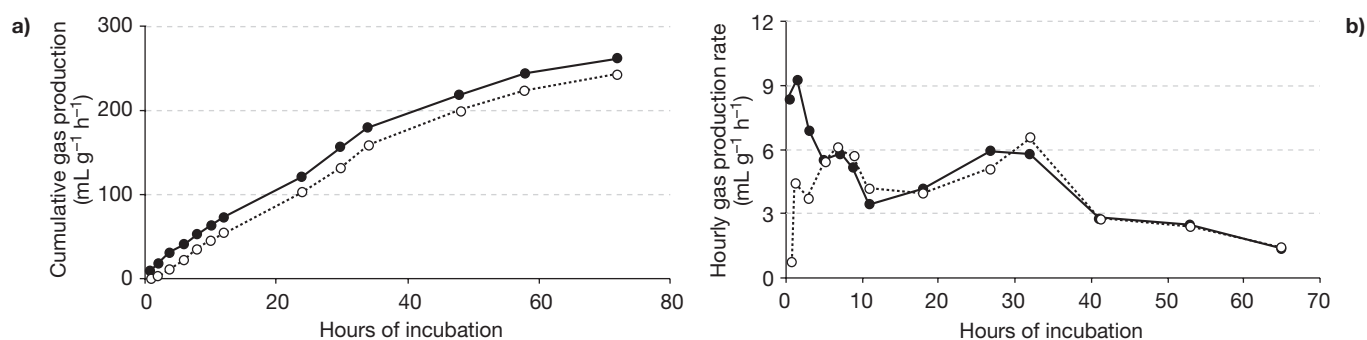


Figure 2. (a) Cumulative and (b) hourly gas production rate of meadow silages stored in field (full line) or under shelter (dotted line).

rates of gas production (later GP rate differences between treatments were non-significant, $P > 0.15$).

In agreement with the chemical composition, fermentation products also showed effects associated with the form of storage, as pH and N-NH₃ (expressed as % of total N, TN) were higher for the silage obtained from minisilos kept in the Field (respectively, Field: 5.6 and 12% and Shelter: 4.4 and 6.8 %, $P < 0.01$; Table 1). Both variables presented a similar pattern (Fig. 3), indicating a loss of acidity beyond 111 d of fermentation in the Field treatment. For both variables, differences between treatments only became apparent as fermentation progressed.

Fermentation acids concentration were similar for both treatments (Table 1), and although the lactic/acetic ratio indicated a strong preponderance of lactic fermentation, a reduction of this ratio over time (15 to 5 g kg⁻¹ DM declining in 230 d) was observed due to the increment in acetic acid concentration.

Discussion

Temperature regime was within the normal range observed in the region, and forage quality was similar to that reported for temperate grasses in terms of CP,

nNDF_{OM} and ADF_{OM}, but the content of ADL_{SA} was substantially lower (26 g kg⁻¹ DM in this work) (Jaurena and Danelón, 2006). The CP contents in the fresh material and silage (*c.a.* 110 g kg⁻¹ DM) presented a slight increase without biological importance between harvest and t₃ sampling (236 d), and was comparable to previous reports for meadows with dominance of *Carex* sp. (Cabeza and Livraghi, 2004).

The WSC contents in the original forage (64 g kg⁻¹ DM) was within the minimum (0-80 g kg⁻¹ DM) suggested for grasses for a proper silage fermentation according to Woolford (1984), but below the threshold of 7 g kg⁻¹ fresh matter suggested by Haigh (1990), hence it can be argued that the forage sugar content could have reduced the ensiling fermentation. Rapid reduction of internal temperatures during the first three days of storage indicated a rapid limitation of respiratory activity brought about by the proper sealing of the minisilos. *In-silo* DM losses (-0.2% and 22% respectively, for Shelter and Field treatments) were in agreement with other authors reports (25%, Bastiman and Altman, 1985; 18%, Jaurena and Pichard, 1999; 3-25%, McDonald *et al.*, 1991; 22%, Moore and Kennedy, 1994; 0-43%, Ruppel *et al.*, 1995; 16%, Watson and Nash, 1960), and clearly indicated the technical convenience of the Shelter treatment.

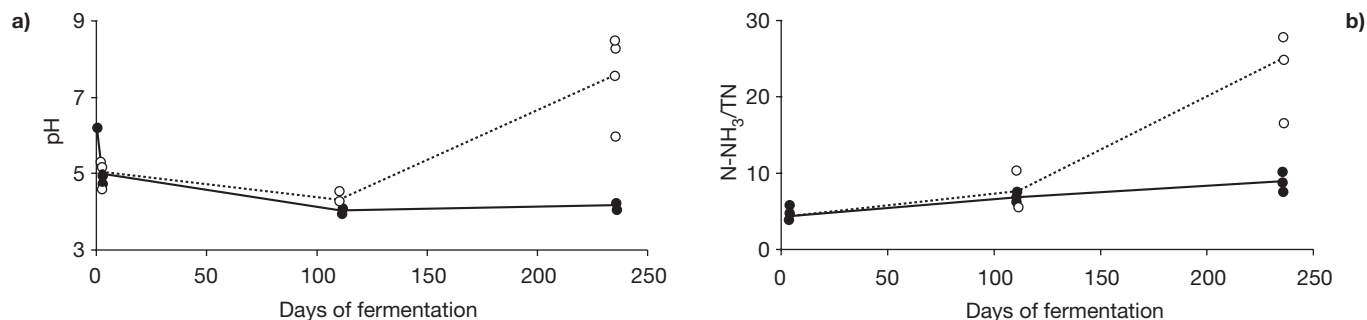


Figure 3. (a) pH and (b) ammonia nitrogen (% total nitrogen) of meadow silages stored in field (full line) or under shelter (dotted line).

During storage, disappearance of non-structural organic matter components induced the increments in ADL_{SA} and ash concentrations, which is in concordance with the above discussed DM losses. Furthermore, $aNDF_{OM}/ADL_{SA}$ and Hemi/ ADL_{SA} ratios signaled a similar pattern between treatments, *i.e.* structural carbohydrates were not affected by treatments, but by t_3 some remotion of Hemi was detected for both. Consequently, it is apparent that $aFDN_{OM}$ and Hemi reduction by t_3 (on a DM basis) in Shelter treatment was probably brought about by the differential disappearance of non-structural components between treatments.

Ensiling forage under shelter led to better fermentation of material as indicated by pH (4.03 at t_2) and N-NH₄ concentration, on the contrary forage samples taken from outdoors minisilos did not reach stability, as indicated by the higher pH and N-NH₄ concentration and beyond 111 d they suffered a drastic increase in acetic acid concentration, pH and proteolysis. The rise in acetic acid concentration could have been associated with the activity of yeasts or acetic bacteria that tend to start the process of deterioration in many silages when exposed to air (Driehuis *et al.*, 1999). However, the production of acetic acid has also been reported from the fermentation of lactic acid by *L. buchneri* at pH as low as 3.8, or *L. bifementans* at pH above 4 (Driehuis *et al.*, 1999), and by propionic bacteria that can proliferate in silages with pH above 4.8 (Weinberg and Muck, 1996). Indeed, future research about ensiling natural forages in this environment will require of a complete description of microbial populations.

Overall, ensiling under shelter contributed to achieve a better stabilization of the silage without apparent *ivDMD* superiority, in agreement with previous results showing that fermentative profile is not necessarily associated with digestibility (Haigh, 1995). This difference was probably associated with the preservation of fermentable carbohydrates in forage ensiled under shelter, as suggested by the gas production yield during the early hours of *in vitro* incubation.

As final conclusions, the conservation of natural forage as silage is a promising alternative for «Tierra del Fuego» livestock production systems. This work intended to assess the feasibility of ensiling forage from natural meadows under two systems with contrasting degrees of protection from the environment, considering that the temperature could constrain microbial development during the ensiling process.

The original quality of the forage despite the advanced degree of maturity at harvest was acceptable

for ensiling and comparable to that obtained in others regions of the country. With respect to the ensiling process, these preliminary and exploratory results are consistent with the hypothesis tested, as forage ensiled under shelter developed a better fermentation, nutritive quality descriptors (ME concentration) and reached stability. On the contrary, forage ensiled in minisilos exposed to the outdoors environmental conditions did not reach proper stability.

These results support the idea that ensiling could play a key role in enhancing livestock production systems, and that local research on this issue is imperative.

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