

Chemical composition and in vitro ruminal degradation of hay and silage from tropical grasses

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Arroquy, J. I., Cornacchione, M. V., Colombatto, D. and Kunst, Jr., C. 2014. **Chemical composition and in vitro ruminal degradation of hay and silage from tropical grasses.** *Can. J. Anim. Sci.* **94**: 705–715. This study was conducted to evaluate the effects of preservation type on chemical composition and in vitro ruminal degradation of warm-season grasses (WSG). Treatments consisted of two factors (6 × 2): the first factor was tropical grasses: *Cenchrus ciliaris* (cv. Biloela, and cv. Molopo), *Chloris gayana* (cv. Callide and cv. Finecut), *Panicum maximum*, and *Brachiaria bryzanta*; and the second factor was preservation type (hay vs. silage). Cell wall, hemicellulose, cellulose, and water-soluble carbohydrate ($P < 0.05$) concentrations were different among WSG. In general, hay or silage altered fiber content compared with fresh. For instance, hemicellulose and cellulose contents were lower in silage than in hay and fresh grass ($P < 0.05$). Gas production rates were higher in silage from 0 to 24 h of fermentation, except at 4 h of incubation. After 24 h, gas production (GP) rate was similar for both preservation types, whilst potential GP was similar between preservation types. However, silage had decreased lag time compared with hay ($P < 0.01$). Silage had greater dry matter disappearance than hay ($P < 0.05$), and gas production yield was similar for grass species and preservation type. Our results indicate that WSG conserved as silage showed beneficial changes in chemical composition and dry matter degradation compared with hay.

Key words: Silage, hay, warm season grasses, gas production, chemical composition

Arroquy, J. I., Cornacchione, M. V., Colombatto, D. et Kunst, Jr., C. 2014. **Composition chimique et dégradation ruminale in vitro du foin et de l'ensilage provenant d'herbes tropicales.** *Can. J. Anim. Sci.* **94**: 705–715. Cette étude a été effectuée pour évaluer les effets du type de conservation sur la composition chimique et la dégradation ruminale in vitro des herbes tropicales de la saison chaude (WSG – « warm-season grasses »). Les traitements consistent de deux facteurs (6 × 2) : le premier facteur est l'herbe tropicale : *Cenchrus ciliaris* (cv. Biloela et cv. Molopo), *Chloris gayana* (cv. Callide et cv. Finecut), *Panicum maximum*, et *Brachiaria bryzanta*; le deuxième facteur est le type de conservation (foin c. ensilage). Les concentrations de parois cellulaires, d'hémicellulose, de cellulose et d'hydrates de carbone hydrosolubles (WSC – « water-soluble carbohydrates ») étaient différentes ($P < 0,05$) parmi les WSG. De façon générale, le foin ou l'ensilage avaient un contenu en fibres altéré par rapport aux herbes fraîches. Par exemple, les contenus en hémicellulose et en cellulose étaient plus faibles dans l'ensilage que dans le foin et les herbes fraîches ($P < 0,05$). Les taux de production de gaz étaient plus élevés dans l'ensilage de 0 à 24 h de fermentation, sauf au temps 4 h d'incubation. Après 24 h, le taux de production de gaz (GP – « gas production ») était similaire pour les deux types de conservation, tandis que la GP potentielle était similaire entre les types de conservation. Par contre, l'ensilage avait un décalage réduit par rapport au foin ($P < 0,01$). L'ensilage avait une plus grande disparition de matières sèches (DMD – « dry matter disappearance ») que le foin ($P < 0,05$), et le rendement en production de gaz (RGY – « gas production yield ») était similaire entre les espèces et les types de conservation. Nos résultats indiquent que les WSG conservées sous forme d'ensilage démontrent des changements bénéfiques dans la composition chimique et la décomposition des matières sèches par rapport à la conservation sous forme de foin.

Mots clés: Ensilage, foin, herbes de la saison chaude, production de gaz, composition chimique

Forage is the most abundant and economical source of feed for ruminants in tropical and subtropical grazing systems (Van Soest 1994). In the dry Chaco of Argentina, cultivated perennial C₄ grasses are important sources of feed for beef-cattle grazing systems (Nasca 2007). Warm-season grasses (WSG) have a well-defined seasonal growth occurring during late spring, full summer, and early fall (Guzmán et al. 1994; De León et al. 1995). This growing season is followed by 5 to 6 mo of dry winter when pasture growth is null. Even though herbage

accumulated in summer and early fall can be deferred to winter, forage quality decreases dramatically in response to the advance into the dry season (De León et al. 1995;

Abbreviations: A, potential gas production; ADL, acid detergent lignin; ADF, acid detergent fiber; CP, crude protein; DM, dry matter; DMD, DM disappearance; GP, gas production; L, lag phase; μ , fractional rate of gas production; NSC, non-structural carbohydrate; NDF, neutral detergent fiber; RGY, gas production yield; T/2, time at which half of the potential gas production is achieved; WSC, water-soluble carbohydrates; WSG, warm-season grasses

Sollenberger et al. 2004). For this reason, to maintain carrying capacity and animal performance throughout the year, it is necessary to conserve forage to be utilized during winter. The most widely used method of forage conservation in grazing systems is hay. Nevertheless, several factors impose important limitations to making good-quality hays (Harrison et al. 2003). In addition, some WSG commonly have a relative high stem-to-leaves ratio at harvest, which extends field drying time, increasing the probability of nutrient loss due to rain (Taliaferro et al. 2004). Compared with hay, silage is easier to handle and reduces rain damage and field losses (Wilkinson 1983). However, chemical composition and morphology make WSG more difficult to ensile compared with annual summer crops (i.e., corn or sorghum) or cool-season grasses. Their low dry matter and water-soluble carbohydrate (WSC) contents, as well as their buffering capacity (Bernardes et al. 2005; Coan et al. 2007), limit silage fermentation and the subsequent adoption of silage technology for these species (Spitaleri et al. 1995). However, nutrient and morphological transformations of roughage produced by respiration, physical loss, and leaching during hay curing (Savoie 1988) are often higher in hay than in silage (Collins 1991). For example, N transformation during baling or ensiling are different, and rate of dry matter (DM) and protein degradability in cool-season grass silages have been observed to be higher compared with hay (Petit and Flipot 1992; Kohn and Allen 1995). Many studies (Adesogan et al. 2004; Dean et al. 2005; Vendramini et al. 2010) have reported lower cell wall concentration in WSG silage compared with fresh forage. In this regard, Jones et al. (1992) suggested that during the ensiling process, cellulases and hemicellulases synthesized by naturally occurring microorganisms in the plant might foster changes in cell wall structure. Then, hydrolases activity during silage fermentation might result in significant changes in structural polysaccharide pools affecting fiber concentration and digestibility. Although various studies (Krishnamoorthy et al. 1995; González Ronquillo et al. 1998) have evaluated the kinetics of degradation in tropical grasses, to our knowledge, no research has been accomplished to compare the effect of the type of conservation method on WSG nutritional value. Our hypothesis was that the silage would affect the kinetics of ruminal DM disappearance in WSG with regard to hay, due to changes in cell-wall structure, and it would be dependent on the WSG species considered. For instance, differences in initial chemical composition between grasses and preservation type would influence in vitro DM degradation in a different way. Thus, the aim of this study was to evaluate the effect of preservation type (hay or silage) on in vitro DM degradation, gas production kinetics, and chemical composition of WSG species.

MATERIALS AND METHODS

Six warm-season grasses seeded in November 2006 at the Experimental Station of the Instituto Nacional de

Tecnología Agropecuaria "La María" (Santiago del Estero, Argentina; lat. 28°3'S, long. 64°15'W) were harvested after 61 d of growth (from January to March of 2008). All forage plots were harvested (8.4 m²) at 12 cm above the ground level. Growing climatic conditions in the region had a well-defined dry winter season, and a hot and humid summer season (631 ± 160 mm average annual precipitations from 1981 to 2007). Average maximum temperatures are 32.4, 31.1, and 28.3°C, and minimum temperatures are 20.1, 19.6, and 18.2°C for January, February, and March respectively (year series from 1997 to 2007). Soil traits at the experimental site were: 0 to 20 cm, pH = 7.7, total organic matter = 1.8%, total N = 0.1%, and P = 95 ppm. Treatments consisted of a 6 × 2 factorial arrangement within a complete randomized block design with three blocks. The first factor consisted of six tropical grasses (main plot): *Cenchrus ciliaris* (cv. Biloela, and cv. Molopo), *Chloris gayana* (cv. Callide, and cv. Finecut), *Panicum maximum* (cv. Gatton panic), and *Brachiaria bryzanta* (cv. Marandú). The second factor tested two preservation types: hay vs. silage. Preservation type factor was nested within grasses (subplot). Small-scale silos and bales were made with three replications per grass. Half of the fresh material from each main plot (randomly selected) was ensiled in small-scale silos and the other half was packed in small-scale bales. Before ensiling, two sample of fresh material were either frozen or dried in an air-forced oven at 55°C, and kept for chemical analysis.

Ensiling Process

Immediately after harvest, 2 h wilted material was chopped through a mill. Chopped forage was then packaged in 500 mm long by 110 mm diameter cylinders to a standard density of 800 kg of fresh material per cubic meter. After 90 d of storage, cylinders were opened. A homogenized aliquot per cylinder was taken to dry in air-forced oven at 55°C for laboratory analysis. Two additional subsamples were taken for pH determination, while the second subsample was frozen for non-fiber carbohydrates and lactic acid analysis.

Baling Process

Fresh forage was left on the plot until the material reached a DM content appropriate for baling. During this field-drying process there was no rainfall, and the material was sun dried during 4 d after clipping. The sun-dried forage was then packed into plastic baskets (500 mm long by 310 mm wide by 110 mm high; mesh size = 10 mm) so that the forage density achieved in the small-scale bales was similar to that in commercial bales (ca., 120 kg DM hay m⁻³). Bale samples were taken on day 90 from harvest, and then were oven dried at 55°C for further laboratory analysis.

Laboratory Analysis

Partially dried samples of fresh material, silage, and hay were dried for 12 h at 105°C for DM determination.

All samples were analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) by sequential analysis with the ANKOM-Fiber Analyzer 200 (ANKOM Technology, Macedon, NY) using the procedure described by Komarek (1993). Sodium sulfite was not used in the NDF analysis, but heat-stable amylase was used during sample reflux and filtering to ensure complete starch solubilization. The NDF and ADF values reported contain residual ash. Crude protein was analyzed using Kjeldhal for the determination of N (Association of Official Analytical Chemists 1980). Frozen samples of fresh and silage material were defrosted at refrigerator temperature, then homogenized and ground in a laboratory mill for determination of WSC by the method proposed by McDonald and Henderson (1964). Total starch was analyzed as described by McCleary et al. (1994). Twenty-five grams of fresh sample (i.e., not frozen) was soaked with 250 mL of deionized water in a refrigerator overnight, and then filtered. A 50 mL aliquot was taken to record silage pH according to Payne and McDonald (1966). Additionally, silage samples were analyzed for lactic acid (Barker and Summerson 1941).

In Vitro Ruminal Fermentation

Gas production along with in vitro incubation was measured using the pressure technique (Mauricio et al. 1999) for hay and silage grass samples. Approximately 500 mg of DM per sample was weighed in quadruplicate and added to 125-mL fermentation bottles. Fermentation medium was composed of 90 mL of anaerobic buffer and 10 mL ruminal fluid. Ruminal bacterial inoculum was prepared by collecting ruminal fluid from two ruminally fistulated beef steers fed alfalfa hay ad libitum. Ruminal fluid was strained through four layers of cheesecloth under CO₂ and kept at 39°C in a water bath. The bacteria-rich fluid to be used as inoculum was mixed with the buffer under CO₂. The fermentation medium was dispensed in 100-mL aliquots into 125-mL bottles, sealed with butyl rubber stoppers, and crimped with aluminum seals. Incubation proceeded for 120 h at 39°C. Head-space gas produced (GP) from substrate fermentation was measured as pressure (psi) at 2, 4, 8, 12, 24, 36, 48, 72, 96, and 120 h of incubation. Pressure values, corrected by the amount of DM substrate incubated, were utilized to estimate gas volumes using the quadratic equation reported by Mauricio et al. (1999). Estimates of GP rate were calculated from the values obtained at each measurement time. Gas values per treatments were averaged across bottles within replicates (i.e., plots) to fit the model. The model of France et al. (1993) was used to describe cumulative GP and degradation profiles, in terms of time-dependent fractional rate of GP (μ), lag phase (L), potential GP (A), and time at which half of the potential GP is achieved (T/2).

At the end of incubation (120 h), dry matter disappearance (DMD) was estimated, and gas accumulated at

this point was related to DMD to calculate relative gas yield (RGY, milliliters of gas per gram of DMD).

Statistical Analysis

The data of forage yield, chemical composition (CP, NDF, ADF, hemicellulose, cellulose, total starch, WSC), DMD, RGY and gas production parameters from the model were analyzed using the MIXED procedure of SAS software (SAS Institute Inc., Cary, NC). Grass species (main plot) and preservation type (subplot) were considered both as fixed effects, whereas block was considered as a random effect of the model. The statistical model was:

$$y_{ijk} = \mu + b_i + g_j + s_k + (gs)_{jk} + \varepsilon_{ijk}$$

where y_{ijk} is the response for block i in WSG j and forage type k ; μ is the overall mean; b_i is a random effect of block i ; g_j is a fixed effect of WSG j ; s_k is a fixed effect of preservation type k ; $(gs)_{jk}$ is a fixed effect of the interaction between WSG treatment j with preservation type treatment k ; and ε_{ijk} is random error.

Data from cumulative gas production and fractional gas production rates at different incubation times were analyzed using the MIXED procedure of SAS. Terms in the model were block, WSG, preservation type, WSG \times preservation type, WSG \times incubation time, preservation type \times incubation time, WSG \times preservation type \times incubation time. The statistical model was:

$$y_{ijkl} = \mu + b_i + g_j + s_k + t_l + (gs)_{jk} + (gt)_{il} + (st)_{kl} + (gst)_{jkl} + \varepsilon_{ijkl}$$

where y_{ijkl} is the response for block i in WSG j and preservation type k at time l ; μ is the overall mean; b_i is a random effect of block; g_j is fixed effect of WSG j ; s_k is a fixed effect of preservation type k ; t_l is a fixed effect at time l ; $(gs)_{jk}$ is a fixed effect of the interaction between WSG treatment j with preservation type treatment k ; $(gt)_{il}$ is a fixed effect of the interaction between WSG j with time l ; $(st)_{kl}$ is a fixed effect of the interaction between preservation type treatment k with time l ; $(gst)_{jkl}$ is a fixed effect of the interaction between WSG j with preservation type treatment k at time l . Treatment mean differences were determined using the LSMEANS procedure. Differences among means were declared significant at $P < 0.05$, whereas trends were discussed at $P < 0.10$.

RESULTS

Harvested Forage

Forage DM yield and plant fractions at harvesting are shown in Table 1. Dry matter concentration did not differ ($P = 0.49$) among WSG. Dry matter contents (data not shown) among grasses ranged from 211 to 244 g kg⁻¹. Accumulation of DM at harvest was similar among WSG, except for *P. maximum*, which had the greatest DM yield (Table 1). *Panicum maximum* had greater stem and lower leaf proportions at harvesting than the other

Table 1. Forage yield and plant fractions at harvest

	Warm-season grasses						SEM	P value
	<i>C. ciliaris</i> cv. Biloela	<i>C. ciliaris</i> cv. Molopo	<i>C. gayana</i> cv. Callide	<i>C. gayana</i> cv. Finecut	<i>P. maximum</i>	<i>B. bryzanta</i>		
DM yield (kg ha ⁻¹ DM)	4124a	4662a	3031a	2277a	8104b	4095a	1731	0.02
<i>Plant fractions</i>								
Stem (g kg ⁻¹ DM)	474b	436b	414b	470b	661a	414b	38	<0.01
Green leaf (g kg ⁻¹ DM)	364b	488ab	516ab	382b	205b	567a	63	<0.01
Dead matter (g kg ⁻¹ DM)	83a	48ab	35ab	40ab	12b	11b	25	0.16
Inflorescence (g kg ⁻¹ DM)	80abc	28bc	36abc	109ab	123a	9c	38	0.11

a-c Values with different letters in the same row differ significantly ($P < 0.05$).

WSG ($P < 0.05$; Table 1). In contrast, *B. bryzanta* and *C. gayana* Callide had lower stem proportion. *Brachiaria bryzanta* had a higher proportion of green leaves ($P < 0.05$), even though it did not differ from *C. ciliaris* Molopo and *C. gayana* Callide. Dead matter ($P = 0.16$) and inflorescence ($P = 0.11$) proportions did not differ among WSG.

Chemical Composition of Fresh Grass, Hay, and Silage

Preservation type (fresh, hay, and silage) \times WSG interaction did not affect CP ($P = 0.49$; data not shown), NDF ($P = 0.19$), ADF ($P = 0.14$), ADL ($P = 0.93$), total starch ($P = 0.19$), and WSC ($P = 0.89$), except for hemicellulose ($P < 0.01$) and cellulose ($P = 0.03$) concentration. There was an effect of WSG on NDF, ADF, ADL, hemicellulose, cellulose, and WSC ($P < 0.05$; Table 2). Starch tended to be different among WSG ($P = 0.08$). Averaging among fresh, hay and silage forage, *B. bryzanta*, *C. ciliaris* Biloela and Molopo and *P. maximum* were similar in NDF, whereas *C. gayana* cultivars had a higher NDF concentration than the other WSG ($P < 0.05$). *Chloris gayana* Finecut did not differ from *C. ciliaris* Molopo in NDF. The lowest content of ADF was observed in *B. bryzanta*, but it was similar to *C. ciliaris* Biloela and *C. gayana* Finecut, while *C. ciliaris* Molopo, *C. gayana* Callide and *P. maximum* showed the highest ADF values ($P < 0.05$). The other grasses showed intermediate values for ADF. Acid detergent lignin was lower for *B. bryzanta* ($P < 0.05$), but this grass did not differ from *C. gayana* Finecut and Callide. *Cenchrus ciliaris* Molopo had a higher ADL content than the other WSG ($P < 0.05$), and *C. ciliaris* Biloela and *P. maximum* were similar to *C. gayana* Callide. Hemicellulose was lower ($P < 0.05$) in *P. maximum* than the other WSG, except when it was compared with *C. ciliaris* Molopo, whereas, hemicellulose concentration was similar between *C. ciliaris*, Molopo and Biloela. *Chloris gayana* Finecut and Callide did not differ between them in hemicellulose, and Callide was also similar to *C. ciliaris* Biloela and *B. bryzanta*. The lowest concentration of cellulose ($P < 0.05$) was observed in *C. ciliaris* Biloela and *B. bryzanta*; meanwhile *C. gayana* Callide had the highest ($P < 0.05$)

concentration of cellulose. The other WSG showed intermediate values. However, there was an interaction with regard to conservation methods (i.e., fresh grass, hay, and silage) and WSG for hemicellulose ($P < 0.01$) and cellulose ($P = 0.03$). The content of hemicellulose in silage was lower ($P < 0.05$) than in fresh grass for *C. gayana* Callide and *B. bryzanta*, while conservation method did not differentially affect hemicellulose in *C. ciliaris* Biloela and Molopo, *C. gayana* Finecut, and *P. maximum* (data not shown). Cellulose in hay did not differ from fresh grasses. In contrast, silage cellulose was significantly lower compared with hay ($P < 0.05$). Additionally, in silage, cellulose decreased more in *C. ciliaris* Biloela and Molopo, *C. gayana* Finecut, *P. maximum*, *B. bryzanta*, than in *C. gayana* Callide (data not shown). Total starch was similar among WSG ($P = 0.08$). With respect to WSC, *B. bryzanta* showed more ($P < 0.05$) concentration than the other grasses.

Silage and fresh grass DM were similar ($P > 0.05$), but lower than hay ($P < 0.05$, Table 3). Hay and silage showed similar CP, and fresh grass had lower CP concentration ($P < 0.05$) than hay, but similar to silage. Hay had higher concentration of NDF and ADF ($P < 0.05$) than fresh grass and silage. While fresh grass NDF was higher ($P < 0.05$) than in silage, ADF did not differ between fresh and silage. The concentration of ADL was higher ($P < 0.05$) in silage compared with fresh grass and hay. Hemicellulose and cellulose contents were lower ($P < 0.05$) in silage than in hay and fresh grass, except for cellulose in fresh grass that did not differ either from hay or silage. During baling and ensiling of grasses, total starch and WSC concentration decreased ($P < 0.05$) approximately onefold compared with fresh grass (Table 3). Hay and silage were similar in total starch and WSC ($P > 0.05$).

pH and Lactic Acid Concentration in Silage

Silage pH did not differ among WSG ($P = 0.61$; Table 4). The concentration of lactic acid in the ensiled material was greater in *B. bryzanta* than in the other grasses (Table 4, $P < 0.05$).

Table 2. Chemical composition for warm-season grasses

Nutrient ^z	Warm-season grasses								SEM	P value
	<i>C. ciliaris</i> cv. Biloela	<i>C. ciliaris</i> cv. Molopo	<i>C. gayana</i> cv. Callide	<i>C. gayana</i> cv. Finecut	<i>P. maximum</i>	<i>B. bryzanta</i>				
CP (g kg ⁻¹ DM)	96.0	89.9	88.5	93.6	86.2	89.2	5.2	0.79		
NDF (g kg ⁻¹ DM)	704a	716ab	747c	739bc	716a	701a	8.8	<0.01		
ADF (g kg ⁻¹ DM)	379ab	402bc	407c	385abc	405c	367a	8.4	<0.01		
ADL (g kg ⁻¹ DM)	60.0c	74.3d	53.2abc	47.5ab	58.6bc	46.1a	4.2	<0.01		
Hemicellulose (g kg ⁻¹ DM)	32.5bc	314ab	340cd	354d	306a	335c	6.0	<0.01		
Cellulose (g kg ⁻¹ DM)	320a	328ab	354c	337bc	347bc	321a	7.6	<0.05		
Starch (g kg ⁻¹ DM)	21.7	24.1	15.5	16.3	21.7	19.0	2.2	0.08		
Water-soluble carbohydrate (g kg ⁻¹ DM)	14.9a	11.6a	12.5a	16.0a	16.3a	21.4b	1.8	<0.01		

^zCP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin. *a-d* Values with different letters in the same row differ significantly ($P < 0.05$).

DM Disappearance and in vitro Gas Production in Hay and Silage

Three- and two-way interactions (i.e., WSG × storage × time, WSG × time, storage × time, WSG × storage) were not detected ($P > 0.26$) for total GP from hay and silage fermentation of WSG (Fig. 1). Gas production increased over time ($P < 0.01$), whereas WSG and storage did not affect GP. Rates of GP were not affected by WSG × storage × time interaction as well as WSG × time and WSG × storage interactions (Fig. 2). However, there was a storage × time interaction ($P < 0.01$) on GP rate. On average, GP rate was initially (2 h) greater for silage than hay ($P < 0.05$; Fig. 3), but at hour 4 of incubation GP rate was higher for hay than silage ($P < 0.05$). Thereafter, GP rate was greater for silage than hay at 8, 12, and 24 h ($P < 0.01$). Mean rate of GP, in both preservation types, had two distinctive peaks at 4 and 24 h of incubation, and then decreased from 24 to 120 h. Gas production rate did not differ between preservation types after 24 h of fermentation.

Fitted parameters of the model are shown in Tables 5 and 6. The WSG × storage interaction was not statistically significant ($P > 0.49$) for potential GP (A), lag phase (L), T/2, or μ at T/2, 12, 24, and 48 h respectively. Potential gas production (A) and T/2 did not differ among WSG, whereas L was shorter in *B. bryzanta* than in *C. ciliaris* Biloela and Molopo, *C. gayana* Callide and Finecut ($P < 0.05$; Table 5), and *P. maximum* showed an intermediate value. There was an effect on μ at T/2, and 48 h ($P < 0.05$) among WSG. Rate at T/2 was greater ($P < 0.05$) for *C. ciliaris* Biloela and *C. gayana* Callide, while *B. bryzanta* and *P. maximum* had the lowest ($P < 0.05$) rate at T/2. An intermediate rate at T/2 was observed in *C. ciliaris* Molopo. *Cenchrus ciliaris* Biloela and Molopo, as well as *C. gayana* Callide and Finecut, had a similar rate at 48 h, but higher than *P. maximum* and *B. bryzanta* ($P < 0.05$). Nonetheless, *C. ciliaris* Molopo and *C. gayana* Callide and Finecut did not differ from *P. maximum* ($P < 0.05$), and the rate at 48 h of *C. gayana* Finecut, *C. ciliaris* Molopo, as well as *P. maximum* were similar to *B. bryzanta*.

Potential GP was similar between preservation types (Table 6). Silage had lower L and T/2 than hay ($P < 0.05$). The rate at T/2 tended ($P < 0.08$) to be higher for silage than for hay. Hay had a lower rate at 12 and 24 h of incubation than silage ($P < 0.05$), but they were similar at 48 h. Dry matter disappearance was higher for *C. gayana* Callide and *B. bryzanta* than for *C. ciliaris* Molopo ($P < 0.05$), but did not vary from *C. ciliaris* Biloela, *C. gayana* Finecut, and *P. maximum*. In silage, DMD was higher than in hay ($P < 0.05$). Relative gas production (RGY) was similar for grass species and silage vs. hay.

DISCUSSION

Forage quality of warm-season grasses remains relatively stable from vegetative to early stages of stem elongation (Jones and Wilson 1987), and then falls rapidly after ear emergence. In our study, forage was

Table 3. Chemical composition of fresh forage, hay and silage by method of forage conservation

Nutrient ^z	Fresh	Hay	Silage	SEM	P value
DM (g kg ⁻¹ DM)	253a	783b	243a	8	<0.01
CP (g kg ⁻¹ DM)	82.8a	96.4b	92.6ab	4.2	0.05
NDF (g kg ⁻¹ DM)	734b	755c	671a	7	<0.01
ADF (g kg ⁻¹ DM)	384a	403b	387a	5.5	0.05
ADL (g kg ⁻¹ DM)	50.1a	54.9a	65.7b	3.4	<0.01
Hemicellulose (g kg ⁻¹ DM)	350b	352b	284a	4.8	<0.01
Cellulose (g kg ⁻¹ DM)	334ab	348b	322a	6.1	<0.01
Starch (g kg ⁻¹ DM)	34.9b	13.5a	10.8a	1.8	<0.01
WSC (g kg ⁻¹ DM)	28.0b	10.2a	8.2a	1.5	<0.01

^zCP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; WSC, water-soluble carbohydrates. a-c Values with different letters in the same row differ significantly ($P < 0.05$).

harvested after 61 d of growth, in which pastures were in the early reproductive stage with only 2.8 to 12.3% emerged ears. There were differences in dry matter accumulation, which were associated with differences in physiological stages across species and cultivars. For instance, *P. maximum* had on average 1.74 to 3.56 times more forage accumulation than the other WSG, and this was mainly associated with its high proportion of stems. In contrast, DM accumulation of the other WSG was inferior due to a lower proportion of stem and higher proportion of leaves. Although fresh forage from WSG had different partition of dry matter accumulation, DM content was low but similar among them. Low DM content has been reported as a limiting factor for silage fermentation (Muhlbach 1999). However, Harrison et al. (2003) did not observe differences on lactic acid concentration and silage pH when DM fluctuated from 19 to 35%. In our case grass silage pH fluctuated between 4.08 and 4.47, fairly below the maximum recommended pH for grass silage (Harrison et al. 1994; <4.7). Additionally lactic acid concentration was within the suggested values (40–70 g of lactic acid kg⁻¹ DM; Cherney and Cherney 2003).

In fresh forage, cell wall proportion and crude protein were similar to values reported by others (Pérez et al. 1995; González Ronquillo et al. 1998) for a similar stage of maturity. Non-structural carbohydrates (NSC) in our study were around the expected values, the concentra-

tion was similar to 56 and 58 d regrowth in tropical grasses reported by Ribeiro et al. (2002) and Bernardes et al. (2005). Total NSC in fresh forage did not differ among WSG. However, starch concentration was numerically greater in *Cenchrus* sp., *P. maximum*, and *B. bryzanta* than *Chloris* sp. Although few studies have determined starch concentration in WSG, our data agree with Buxton and O'Keily (2003), who suggested that even though its concentration is low, starch is the main source of NSC in WSG. Both, starch and WSC are the main sources of rapidly available energy in the rumen, whereas WSC is the first fermentable substrate for ensiling fermentation (Buxton and O'Keily 2003). Starch in all stored grasses was significantly lower than in fresh forage, which might be related to the fact that highly fermentable carbohydrates were reduced during baling (Coblentz et al. 1997) and ensiling (Owens et al. 1999). Similarly WSC concentrations were also lower in stored than in fresh grasses, and these results are in agreement with previous studies (Harrison et al. 1994; González and Rodríguez 2003; Vendramini et al. 2010).

In our study, CP concentration was similar among species regardless of preservation type (e.g., 94.5 ± 1.5 g kg⁻¹ DM), but was numerically higher than fresh forage. This fact might be due to metabolization of carbohydrate fractions (i.e., respiration in hay or fermentation in silage) during hay wilting or silage fermentation (Van Soest 1994; Bernardes et al. 2005), which indirectly increased 14% CP concentration in stored compared with fresh forage. Crude protein was similar to values reported in the literature (Juárez Reyes et al. 2009). Neutral detergent fiber and ADF, in our case, were in agreement with information reported in the literature (González Ronquillo et al. 1998; González and Rodríguez 2003; Vendramini et al. 2010). For both preservation types, ADF concentration varied among grasses, which might be due to differences in the stage of maturity among species at harvesting. It is well known that there is a negative correlation between ADF and digestibility (Minson 1990). For example, DM digestibility estimated from ADF (Minson 1990) showed that *B. bryzanta* had 11.2% higher DMD compared with *P. maximum*. Lignin is a limiting factor for forage digestibility, particularly for WSG; however, lignin concentration observed in our study was within the normal range for C4 grasses (Minson 1990). Even though there is not necessarily a

Table 4. pH and lactic acid content of warm-season grasses silage

	Warm-season grasses							EEM	P value
	<i>C. ciliaris</i> cv. Biloela	<i>C. ciliaris</i> cv. Molopo	<i>C. gayana</i> cv. Callide	<i>C. gayana</i> cv. Finecut	<i>P. maximum</i>	<i>B. bryzanta</i>			
pH	4.37	4.4	4.47	4.41	4.08	4.34	0.27	0.61	
Lactic acid (g kg ⁻¹ DM)	49.4b	41.6b	39.1b	46.0b	54.8b	76.5a	9.9	<0.01	

a, b Values with different letters in the same row differ significantly ($P < 0.05$).

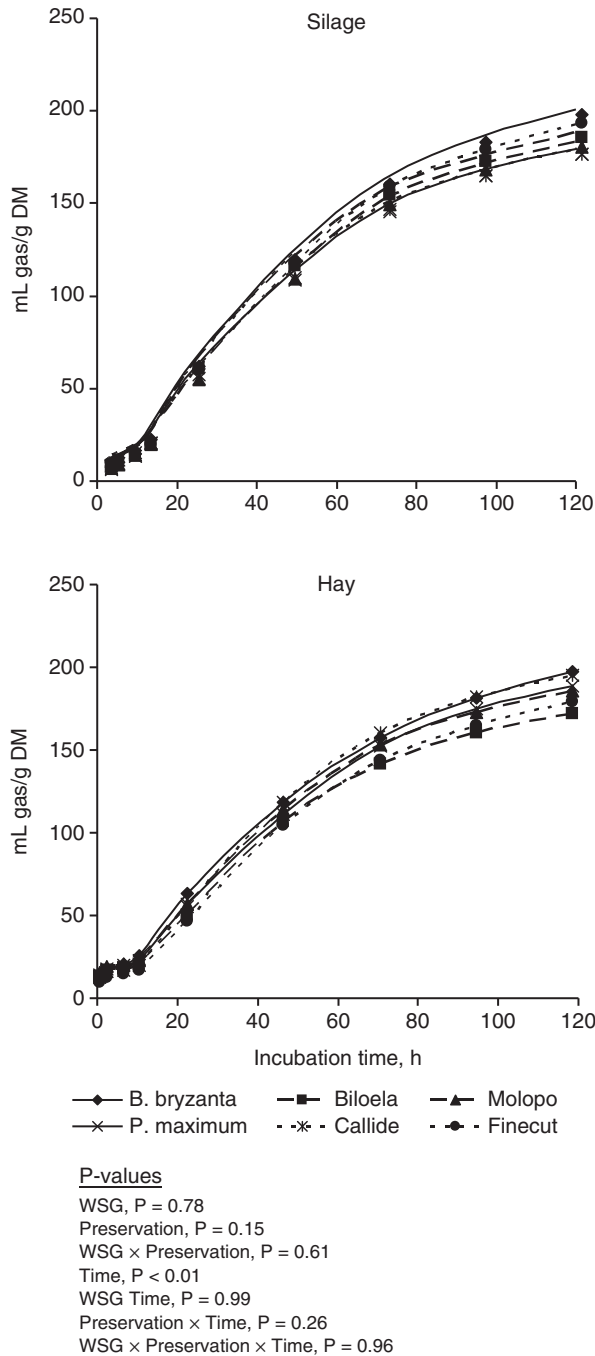


Fig. 1. Pattern of accumulated gas volume produced by in vitro fermentation of silage and hay of warm-season grasses [WSG; Biloela = *Cenchrus ciliaris* (cv. Biloela), Molopo = *Cenchrus ciliaris* (cv. Molopo), Callide = *Chloris gayana* (cv. Callide), Finecut = *Chloris gayana* (cv. Fine Cut), P. maximum = *Panicum maximum* (cv. Gatton panic), B. bryzanta = *Brachiaria bryzanta* (cv. Marandú)]. Standard error of means = 7.63.

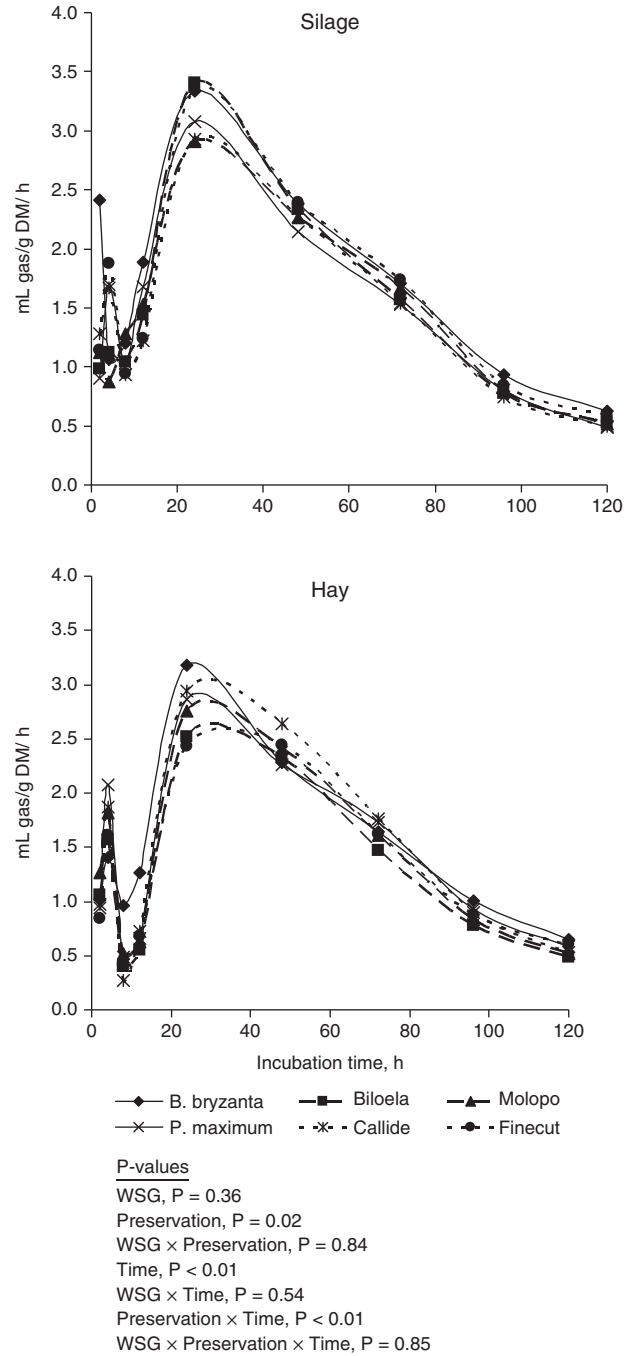


Fig. 2. Rate of gas production over time by in vitro fermentation of silage and hay of warm-season grasses [WSG; Biloela = *Cenchrus ciliaris* (cv. Biloela), Molopo = *Cenchrus ciliaris* (cv. Molopo), Callide = *Chloris gayana* (cv. Callide), Finecut = *Chloris gayana* (cv. Fine Cut), P. maximum = *Panicum maximum* (cv. Gatton panic), B. bryzanta = *Brachiaria bryzanta* (cv. Marandú)]. Standard error of means = 0.26.

clear relationship between chemical composition and microbial degradation as well as with DMI, some studies state that fiber and CP are the main factors related

to digestibility and intake (Vadiveloo and Fadel 1992; Van Soest 1994). In this regard Ford and Elliot (1989) proposed the lignin:hemicellulose ratio as a strong index

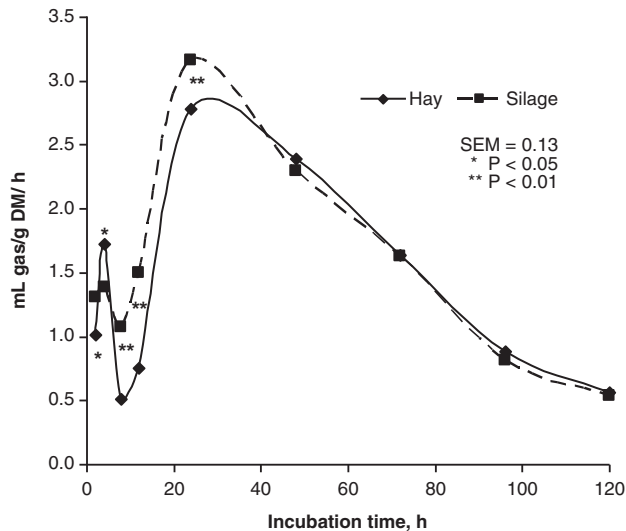


Fig. 3. Average rate of gas production over time by in vitro fermentation of silage and hay of warm-season grasses. Standard error of means = 0.13. * and ** indicate a difference between silage and hay at a given incubation time with a probability of $P < 0.05$ and $P < 0.01$, respectively.

for roughage degradability. Among the grasses evaluated, *B. bryzanta* and *Chloris* sp. (~ 0.14) had a low proportion of lignin:hemicellulose, whereas *Cenchrus* sp. and *P. maximum* had a higher ratio (ca., 0.19 to 0.22). In our case, a higher DMD was expected from *B. bryzanta* and *Chloris* sp. compared with *Cenchrus* sp. and *P. maximum*. However this ratio does not always correspond with DMD, as was observed for *P. maximum*.

Averaged across species, chemical composition showed significant differences between hay and silage. Crude protein and NSC were similar among preservation types, but NDF and ADF were lower in silage than in hay. In both preservation types, WSC and starch were approximately 62 and 70% lower, respectively, than in fresh grass. Chemical values observed in this study are similar to those in previous studies (Muhlbach 1999; Bernardes et al. 2005). In general, tropical grasses have low NSC concentration compared with corn and sorghum (Bernardes et al. 2005). In a study that evaluated the fermentation profile of 60-d Tanzania regrowth (*P. maximum* Jacq. Cv. Tanzania), Silva Cabral et al. (2004) found a small uptake of WSC during the first 30 to 40 d of ensiling, but after 60 d they observed a complete uptake of sugars.

Neutral detergent fiber concentration in silage decreased 8.5%, while ADF did not change with regard to fresh material. In contrast, ADL was concentrated (15.6 g kg⁻¹ DM) in response to ensiling. Cell wall variation was associated with a lower concentration of hemicelluloses and cellulose in silage. Fiber carbohydrates are a potentially degradable fraction during the ensiling process, but are less readily available than NSC. Previous studies have reported a similar reduction in cell wall

during the ensiling process (Jones et al. 1992; Dean et al. 2005; Vendramini et al. 2010). The main changes in cell wall were found in the hemicelluloses fraction (Jones et al. 1992). Low concentrations of NSC in warm-season grasses might foster the use of fiber carbohydrates as an alternative substrate for long-term silage fermentation. Kung et al. (1991) suggested that cell wall degradation in silage might be due to extracellular cellulases and hemicellulases synthesized by microorganisms naturally present in plants. Chamberlain (1987), using *Lolium multiflorum*, found an increase in soluble carbohydrates after 6 and 7 d of ensiling, suggesting that this increase was associated with the breakdown of structural carbohydrates, particularly due to hemicelluloses breakdown. Similarly, we found greater disappearance of hemicellulose ($\sim 18.9\%$ relative to fresh material) than of cellulose ($\sim 3.6\%$ relative to fresh material) when forage was ensiled.

From the standpoint of silage fermentation, NSC and hemicelluloses breakdown might serve as a source of monosaccharides for organic acid production for silage microorganisms. In our study, lactic acid (51.2 ± 9.9 g kg⁻¹ DM) and pH (4.35 ± 0.27) values might support the argument that hemicelluloses, at least in part, contributed as a source of monosaccharide for organic acid production. Silage lactic acid was about 1.3 (i.e., Callide) and 2.55 (i.e., *B. bryzanta*) times the recommended values (30 g kg⁻¹ DM; Weinberg et al. 2008). Adesogan et al. (2004) and Dean et al. (2005) observed lower pH and more lactic acid when exogenous fibrolytic enzymes were applied to Bermudagrass silage. In our experiment, it is possible that naturally existing fibrolytic enzymes might have elicited beneficial changes in silage fermentation.

In contrast, NDF and ADF concentrations in hay were increased compared with fresh material. However hemicelluloses and cellulose for hay were similar to fresh grass. The increase in structural carbohydrates concentration in hay is related to intrinsic NSC hydrolysis, respiration, and aerobic microbial activities during the field-drying process.

The kinetics of ruminal degradation by in vitro gas production technique potentially reflect in vivo digestibility of forages in ruminants (Getachew et al. 2004). It is important to note that in WSG, GP kinetics represents the contribution of cell wall digestibility rather than WSC, because WSG are particularly low in WSC. Digestibility of fiber is an important parameter of forage quality because forage fiber varies in ruminal degradability (Nocek and Russell 1988; Oba and Allen 1999) and cell wall digestibility affects animal performance. Neutral detergent fiber often limits feed intake because of physical filling of the rumen. Then changes in the rate of NDF disappearance affect voluntary feed intake (Dado and Allen 1995; Grant et al. 1995; Oba and Allen 1999). One percentage unit of increment in NDF digestibility (in vitro) elicited a 0.17 kg increase in DMI in Holstein cows (Oba and Allen 1999). Higher NDF

Table 5. Gas production parameters (France et al. 1993), dry matter disappearance, relative gas production, from in vitro fermentation of stored (hay and silage) warm-season grasses

Items	Warm-season grasses						SEM	P value
	<i>C. ciliaris</i> cv. Biloela	<i>C. ciliaris</i> cv. Molopo	<i>C. gayana</i> cv. Callide	<i>C. gayana</i> cv. Finecut	<i>P. maximum</i>	<i>B. bryzantia</i>		
<i>Gas production parameters^z</i>								
A (mL)	189.9	197.2	198.9	202.1	199.1	215.4	20.4	0.43
L (h)	3.96a	3.87a	4.03a	4.00a	3.71ab	3.49b	0.3	0.04
T/2 (h)	40.2	41.3	41.2	43.6	42.7	42.6	3	0.43
μ at T2 (h ⁻¹)	0.0234a	0.0223ab	0.0230a	0.0218ab	0.0212b	0.0208b	0.00046	0.03
μ at 12 h (h ⁻¹)	0.0143	0.0137	0.0137	0.013	0.0136	0.0139	0.0006	0.9
μ at 24 h (h ⁻¹)	0.0202	0.0191	0.0196	0.0184	0.0184	0.0182	0.00056	0.35
μ at 48 h (h ⁻¹)	0.0243a	0.0229abc	0.0238ab	0.0223abc	0.0217bc	0.0213c	0.00056	0.05
DMD ^y (g kg ⁻¹ DM)	574ab	553a	621b	602ab	608ab	621b	19	0.04
RGY ^x (mL g ⁻¹ DMD)	317	333	300	309	301	323	17	0.39

^zL, lag phase; A, potential gas production; T/2, time at which half of the potential gas production is achieved; μ, fractional rate of gas production.

^yDMD, dry matter disappearance.

^xRGY, relative gas production.

a-c Values with different letters in the same row differ significantly ($P < 0.05$).

Table 6. Gas production parameters (France et al. 1993), dry matter disappearance and relative gas production of hay and silage

Items	Hay	Silage	SEM	P value
<i>Gas production parameters^z</i>				
A (mL)	199.3	201.5	20.4	0.75
L (h)	4.06a	3.62b	0.3	<0.01
T/2 (h)	43.5a	40.3b	3	<0.01
μ at T2 (h ⁻¹)	0.0216	0.0225	0.00046	0.09
μ at 12 h (h ⁻¹)	0.0126b	0.0148a	0.0006	<0.01
μ at 24 h (h ⁻¹)	0.0182b	0.0198a	0.00056	<0.01
μ at 48 h (h ⁻¹)	0.0221	0.0233	0.00056	0.06
DMD ^y (g kg ⁻¹ DM)	569b	624a	11	<0.01
RGY ^x (mL g ⁻¹ DMD)	326	302	9	0.28

^zL, lag phase; A, potential gas production; T/2, time at which half of the potential gas production is achieved; μ, fractional rate of gas production.

^yDMD, dry matter disappearance.

^xRGY, relative gas production.

a-b Values with different letters in the same row differ significantly ($P < 0.05$).

degradability allows greater microbial fermentation in the rumen, which increases energy availability of the diet. In our study, accumulated gas production for silage and hay showed similar patterns to those observed by other authors (Gonzalez Ronquillo et al. 1998; Nogueira Filho et al. 2000; Juárez Reyes et al. 2009) with fresh WSG. However, direct comparisons with other experiments are very difficult, since stage of maturity and growing conditions are often very different, and those studies utilized fresh forage rather than hay or silage. When gas production curves are examined, differences between hay and silage were clearly noticed. There were no differences in rate of fermentation among species (Fig. 2), but silage in the first 24 h had a higher rate of gas production. Initially, silage had an advantage, since GP rate was greater in silage than hay during the first 2 h, but at 4 h of incubation GP rate in hay was greater than in silage. The first GP peak might have been produced by WSC, soluble starch fractions and/or soluble fibers. Thereafter, GP rates were greater for silage than hay until 24 h. These findings are supported by the parameters of the model (France et al. 1993). First, a shorter lag phase and a greater fractional rate at 12 and 24 h in silage compared with hay are evident. This was also supported by a reduced time to reach half of the GP asymptote, which was shorter in silage (Table 6). Mean rate of GP, in both preservation types, had two distinctive peaks at 4 and 24 h of incubation, and then decreased from 24 to 120 h. Gas production rate did not differ between preservation types after 24 h of fermentation. This finding is further supported by the lack of differences observed in the fractional rate of GP at 48 h obtained by the model. In an in situ study carried out to compare grass silage to hay, Petit and Tremblay (1992) observed a greater proportion of rapidly degradable fractions in silage than hay. In contrast to these results, the increases in degradation rates at early stages

of incubation (i.e., from 6 to 24 h) in silage compared with hay support the hypothesis that changes in cell wall structure during the ensiling process may facilitate earlier access of ruminal microorganisms to the fiber. This is further suggested by the observed hemicellulose losses during ensiling with respect to fresh forage, and by the greater extension of dry matter disappearance in silage after 120 h of fermentation. The Lag phase and rate of gas production was different among grasses. *Brachiaria bryzanta* had a shorter lag than other species, except for *P. maximum*. Differences observed in lag might be due to lower content of fiber and lignin, as well as higher WSC in stored roughage in *B. bryzanta*. Gas production rates were similar to those observed in previous studies with WSG (Gonzalez Ronquillo et al. 1998; Nogueira Filho et al. 2000). Gas production rates in *C. ciliaris* Biloela and *C. gayana* Callide were higher than in *P. maximum* and *B. bryzanta*, whereas *C. gayana* Finecut and *C. ciliaris* Molopo showed intermediate rates. However, we did not observe any differences in chemical composition that may explain the differences in gas production rates. Non-structural carbohydrates, as well as fiber and lignin concentrations did not show substantial differences that would support the detected differences in gas production rate. But factors related to cell wall arrangement (maturity, stems and leaf proportion) might affect digestion or degradability of the forage, regardless of the actual lignin content (Hatfield 1993).

CONCLUSIONS

This study shows that the method of forage conservation affects dry matter degradation kinetics, dry matter digestibility, and chemical composition of warm-season grasses. Forage quality can be improved by using silage rather than hay for forage conservation. Forage conserved as silage resulted in a greater rate and extent of dry matter digestion compared with hay. It is speculated that silage appears to beneficially alter cell wall structure, allowing for a higher gas production rate than hay during the first 24 h of fermentation, regardless of species evaluated.

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