The nutritive value of five pasture species occurring in the summer grazing ranges of the Pyrenees

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Abstract

Five species of alpine pasture plants from the Pyrenees representing 3 botanical groups : grasses (*Festuca eskia*, *Nardus stricta*), forbs (*Anthyllis vulneraria*, *Galium verum*) and shrubs (*Echinopartum horridum*), were collected monthly from June to September and analysed for nitrogen (N) content, cell wall composition, in vitro enzymatic digestibility (DMDg) and volume of gas produced by microbial fermentation. Among the dicotyledenous varieties, *A. vulneraria* and *G. verum* showed the highest nutritive value whilst that of *E. horridum* was low due to high lignin content. Grasses showed moderate nutritive values in June rapidly decreasing thereafter. Nitrogen content and organic matter digestibility (OMDg) of *A. vulneraria* remained relatively constant through the sampling period whereas it abruptly decreased for remaining species from July. Gas production significantly differed among species during the first 48 h of microbial fermentation but not at later stages of fermentation. Collection date did not affect gas production before 24 h of incubation but significant differences were found thereafter with samples from June and July being more degraded than from August and September. Principal component analysis associated OMDg positively with N content and gas production and negatively with fibre content. Lignin proportion did not significantly correlate with gas production or with OMDg, suggesting that the degree of lignification is not the only factor affecting microbial fermentation but other factors such as lignin tissue locations may be involved. *A. vulneraria* has been revealed as very good forage with a high potential in extensive animal production systems. Both OMDg and DMDg methods seem more accurate than chemical analyses for evaluating forages at different stages of maturity.

Keywords: Alpine grasslands, digestibility, gas production.

Introduction

As in other mountain systems, in the Pyrenees there is an alpine (high altitude) pasture band, generally over 1800 m, that is utilized for livestock grazing. These pastures are spread over 10 000 km² and are an important source of forage for both wild and domestic ruminants. Traditionally, ruminant production in the area is based on grazing alpine pastures only during the summer, whereas from autumn to spring herds are moved down to the valleys, and offered other forage resources (Montserrat and Fillat, 1990). Gómez *et al.* (1997) highlighted the high diversity of Pyrenean high altitude pastures which are composed of more than 500 species. The major proportion of the biomass is

composed of grasses (50 to 60%), such as *Festuca rubra*, *F. eskia*, *F. gautieri*, *Nardus stricta*, whilst legumes such as *Trifolium alpinum*, *Anthyllis vulneraria*, *Lotus alpinus* and forbs such as *Plantago* spp. comprised the remainder of the pasture (approx. 10 to 20% legumes and 5 to 10% forbs).

However, despite the importance of alpine pastures in the livestock system in the Pyrenees, there is a lack of information about the nutritional value of the different species. The botanical group of the species and their phenology are two of the main factors influencing nutritive value. Digestibility of shrubs and grasses are generally lower than legumes and other herbaceous dicotyledenous species. In

addition, the quality of immature growth stages is generally higher than mature stages (Van Soest, 1994). Negative effect of age on the nutritive value of forages is mainly due to a low ratio of leaves to stem (Ughe, 1986) and an increase in stem cell walls and lignification.

This work investigates the effect of plant age on the nutritive value of five pasture species commonly found in Pyrenean pastures and representatives of different botanical groups, selected on the basis of their consumption by the ruminant livestock. In addition, we compare three different methods to estimate the nutritional value of these forages.

Material and methods

The study area consists of a 1200 ha grazing area located in the Western Spanish Pyrenees (Aisa Valley) at an altitude of between 1600 and 2200 m. Pastures were subjected to a moderate grazing pressure of 0.5 to 0.8 LU per ha (1 livestock unit (LU) corresponds to a 500-kg cow) during summer. Local climate is characterized by cold winters and temperate summers and annual rainfall is between 1500 and 2000 mm. Total rainfall during the sampling period was 262.7 mm, and maximum and minimum temperatures ranged between 7.6 and 22.1°C, with an average temperature of 14.8°C. Environmental and animal management characteristics of the studied area are detailed in García-González *et al.* (1990 and 1991).

Plant species were selected on the basis of their abundance in the study area (Gómez *et al.*, 1997). They are also representatives of different botanical groups (grasses, forbs, and shrubs) which correspond to different forage characteristics (Van Soest, 1994). *Festuca eskia* and *Nardus stricta* (grasses) form the major proportion of two of the main phytosociological communities in the Pyrenees: *Festucion eskiae* and *Nardion strictae* (Braun-Blanquet, 1948). *Anthyllis vulneraria* (legume) and

Galium verum (herbaceous dicotyledenous species) are characteristic of *Festucion gautieri* and *Bromion erecti* communities, that are important in the area in terms of surface and herbivore utilization (García-González *et al.*, 1991). *Echinopartum horridum* (a spiny cushion shrub) is an invasive species in Western Pyrenees that has been favoured by pastoral fire and stocking rate diminution, and it is also heavily consumed by browsing ruminants during growing and flowering periods (Aldezabal, 2001). Sampling criteria was that samples would reflect the nutritive conditions of the species as they were found by the grazing herbivores in the study area.

A non-fenced parcel of roughly 1 ha per species, representing average environmental and herbivore utilization conditions, were marked and each species was sampled at monthly intervals from June to September 2000. Except for *E. horridum*, the selected species are difficult to sample in purity because they are frequently mixed with that of other species, and so the leaves and stems of each species were selected and cut by hand. Each sample was composed of different individuals of the same species up to 50 g fresh weight. Samples were taken from ungrazed items, and were clipped simulating the feeding height of herbivores: upper half of unfertile leaves (tiller) for grasses, total stem with their leaves (ca. 10 cm high) for forbs and current annual growth (ca. 5 cm) for the shrub. A description of the phenology for each sample is shown in Table 1. Samples were thoroughly mixed in the laboratory, dried at 60°C for 48 h, and ground to a maximum 1 mm particle size.

The microbial fermentation pattern of collected species was studied by measuring the gas produced during the *in vitro* incubation of samples, following the technique of Theodorou *et al.* (1994). Two fermentation trials were conducted, with duplicate sealed bottles with approximately 800 mg of each substrate in each trial. In addition, triplicate bottles of incubation solution but without substrate were also included as blanks. Rumen contents were obtained from three adult sheep via rumen cannula just before the morning food was given (900 g of a 50:50 barley straw and lucerne hay diet enriched with 20 g of a vitamin-mineral mixture), pooled and used as the main portion of the inoculum solution at a final proportion of 0.10 of the total inoculum volume. Bottles were filled (80 ml) and incubated at 39°C, and the pressure of gas produced in each bottle was recorded by means of a HD8804 manometer with a TP 804 pressure gauge (DELTA OHM) after 2, 4, 6, 8, 12, 24, 36, 48, 60, 72 and 96 h of incubation. Readings were converted into volume (ml) by using the following pre-established linear regression

Table 1 Description of the stage of plant development of the different species when collected on each date

Species	June	July	August	September
<i>Festuca eskia</i>	1	3	5	7
<i>Nardus stricta</i>	1-2	5	7	8
<i>Anthyllis vulneraria</i>	3-4	5	6	7
<i>Galium verum</i>	1-2	3	3-4	8-9
<i>Echinopartum horridum</i>	1	3	7	6-9

1: vegetative growth; 2: budding; 3: flowering; 4: beginning of fruiting, seeding; 5: fruiting, seeding; 6: dispersing seed, fruit; 7: some leaves dried; 8: senescence; 9: regrowth.

between pressure recorded (mbar) in the same type of bottles and known air volumes:

$$\text{volume} = (\text{pressure} - 0.981) / 30.375 \\ (r = 0.996; \text{no.} = 64).$$

The gas volume for each incubation time was expressed per unit of incubated dry matter (DM). Apparent organic matter (OM) digestibility (OMDg, %) of forages was estimated from the volume of gas produced after 24 h of incubation (GP, ml/200 mg DM) and the proportion of crude protein (CP, g/kg DM), following the equation of Menke and Steingass (1988):

$$\text{OMDg} = 24.91 + 0.7222 \text{ GP} + 0.0815 \text{ CP}; \\ \text{no.} = 185; R^2 = 0.78.$$

To estimate the pattern of microbial fermentation, the average cumulative gas production from each forage species and from each sampling date was fitted iteratively to the model proposed by France *et al.* (1993):

$$y = A \{1 - \exp[-b(t - T) - c(\sqrt{t} - \sqrt{T})]\},$$

where y represents the cumulative gas production (ml), t is incubation time (h), A is asymptote (total gas; ml), T is lag time (h) and b and c are rate constants (per h and per $\text{h}^{1/2}$). The maximum proportion of error allowed in the adjustment was fixed at 0.001. The time required to reach one half of the maximum gas production ($t_{1/2}$) was also estimated, and the fractional degradation rate (μ , per h) was calculated at that point (giving $\mu_{1/2}$) according to:

$$\mu_{1/2} = b + c / (2\sqrt{t_{1/2}})$$

In vitro enzymatic digestion (DMDe) was determined following Aufrère and Michalet-Doreau (1988), incubating duplicated samples in two consecutive 24-h phases, firstly with pig stomach pepsin (FLUKA, ref. 77160) and secondly with cellulases from *Trichoderma viridae* (BDH, ref. 39074) at a pH 4.6. Dried plant samples were analysed for their Kjeldahl nitrogen by the Association of Official Analytical Chemists (1990) methods. Neutral-detergent fibre (NDF), acid-detergent fibre (ADF), acid-detergent lignin (ADL) and residual ashes were determined sequentially by the procedures described by Van Soest *et al.* (1991). All analyses were performed in duplicate, and the average values were used for comparing samples. An estimation of DM digestibility (DMDe) of samples was calculated from the chemical composition data, using the equation proposed by Van Soest (1994), considering the

cellular content (CC) digestibility to be: $(0.98 \text{ CC}) - 12.9$ and the NDF digestibility to be: $\text{NDF} [1.473 - 0.789 \log_{10} (\text{ADL} / \text{ADF} \times 100)]$.

Differences among experimental treatments were contrasted by analysis of variance as a 5×4 factorial design, comparing the effect of species, the cutting date and their interaction with the residual. In the *in vitro* gas production studies, the average value of the two bottles from each incubation run was considered to be the experimental unit, and the experimental run was considered as a block. Treatment means were compared by the *post hoc* Tukey's t test at a $P < 0.05$ and orthogonal contrasts were performed. A principal components analysis (PCA) with chemical composition, digestibility and gas production variables was performed. The PCA analysis allows the detection of the degree of association between variables by means of their relative position in a multivariate space, which is reduced to orthogonal directions (axis) of maximum variance in the original data (Afifi and Clark, 1996). In addition, a projection of cases (species-collection-date data) over the same PCA axis was conducted to establish the relationships between cases and variables observing their relative position in the factor space (StatSoft, 1995).

Table 2 Chemical composition† (g/kg dry matter) of plants according to the collection date

Species	Date	N	NDF	ADF	ADL
<i>F. eskia</i>	June	27.8	681	306	57
<i>N. stricta</i>		28.4	749	300	51
<i>A. vulneraria</i>		22.3	454	274	75
<i>G. verum</i>		33.8	316	194	82
<i>E. horridum</i>		24.8	571	354	165
<i>F. eskia</i>	July	14.2	733	367	56
<i>N. stricta</i>		19.2	768	368	77
<i>A. vulneraria</i>		22.2	362	222	65
<i>G. verum</i>		22.8	380	230	107
<i>E. horridum</i>		14.0	633	456	189
<i>F. eskia</i>	August	11.5	640	316	53
<i>N. stricta</i>		13.8	770	359	44
<i>A. vulneraria</i>		21.7	356	158	52
<i>G. verum</i>		22.9	376	233	121
<i>E. horridum</i>		11.8	677	515	215
<i>F. eskia</i>	September	10.9	674	479	60
<i>N. stricta</i>		10.0	777	360	39
<i>A. vulneraria</i>		20.9	293	237	40
<i>G. verum</i>		20.1	377	230	103
<i>E. horridum</i>		12.3	698	490	204
Residual s. d.		3.26	49.4	50.8	16.3
Significance of:					
species effect		**	***	***	***
date effect		***			

† N = nitrogen; NDF = neutral-detergent fibre; ADF = acid-detergent fibre; ADL = acid-detergent lignin.

Results

Chemical composition of the five studied species at each sampling date is shown in Table 2. There were significant differences among species for all variables. The nitrogen (N) content of *G. verum* was higher than *F. eskia* and *E. horridum* ($P < 0.01$). *A. vulneraria* and *G. verum* had the lowest values of NDF and ADF ($P < 0.001$), whereas *E. horridum* and *G. verum* showed the highest lignin content ($P < 0.001$). Only N concentration varied with time ($P < 0.001$), decreasing from June (27.4 g/kg) to other sampling dates (18.4, 16.3 and 14.8 g/kg in July, August and September, respectively). All the species showed this trend, except *A. vulneraria*, whose N content remained fairly constant through the whole experimental period.

When the volume of gas produced from each species on each date by their microbial fermentation *in vitro* was studied, no significant differences for the interaction species $\times$ date were observed at any incubation time. Therefore, the average means of the gas production pattern for each species and each collection date are presented in Figures 1 and 2, respectively. Differences in the volume of gas produced by microbial fermentation were observed among the studied species during the first 48 h of incubation ($P < 0.01$) but not thereafter. During the first 8 h of incubation, *A. vulneraria* showed higher gas volumes than the other species, and differences from *F. eskia*, and with *E. horridum* and *N. stricta* were maintained during the first 36 and 48 h of incubation, respectively. The gas volume produced from *G. verum* was higher than from *N. stricta* at 6 to 36 h, and higher than *E. horridum* at 12 and 24 h. The parameters of the curves fitted to the average gas production pattern for each species are shown in

Figure 1 Average gas production pattern from samples taken from different species (*F. eskia*, Δ ; *N. stricta*, $\circ$; *A. vulneraria*, $\square$; *G. verum*, $\bullet$; *E. horridum*, $\blacksquare$) when incubated *in vitro*, expressed per unit of incubated dry matter (no. = 8). Upper bars show standard error of means.

Figure 2 Average gas production pattern from samples taken from different sampling dates (June, $\blacksquare$; July, $\square$; August, $\bullet$; September, $\circ$) when incubated *in vitro*, expressed per unit of incubated dry matter (no. = 10). Upper bars show standard error of means.

Table 3. Although the fitted parameters were not compared statistically, it can be seen that the maximum gas production (A) from *N. stricta* and *F. eskia* was higher than from the other species. This is in apparent contradiction with the recorded gas volumes at 96 h of incubation, which showed smaller differences (Table 3). This can be explained by the lower fractional fermentation rate of the former species, allowing the asymptote to be reached only after the recorded incubation period had finished, thus forcing the fitted curve to a higher maximum. *A. vulneraria* and *G. verum* were fermented faster than the other species, as shown by their fractional rates of fermentation ($\mu_{1/2}$), and reached half of their potential gas production in 12 and 15 h, respectively, in agreement with the pattern shown in Figure 1. In all cases, lag time was lower than 1 h.

As can be seen from Figure 2, no differences among collection dates were observed until 24 h of fermentation, when samples from June produced more gas than those from September ($P < 0.05$). From 36 h onwards, the stage of maturity of species linearly affected their microbial fermentation ($P < 0.05$), the gas volume of samples from June being higher than August and September, and those from July higher than September. In this case, the estimated maximum gas production (A ; Table 3) was slightly higher than the observed results after 96 h of incubation, but there was a linear reduction of the fractional rate of fermentation as the stage of maturity advanced.

Estimates of digestibility from chemical composition, *in vitro* enzymatic digestion and gas production for species and collection dates are presented in Table 4. There were significant differences in digestibility

Table 3 Coefficients of the curves fitted to the average gas production pattern of each species and on each sampling date, together with the volume of gas recorded after 96 h of incubation (residual s. d. = 17.6; ml/g dry matter (DM))†

	A	b	c	T	$t_{1/2}$	$\mu_{1/2}$	Vol. 96 h
<i>F. eskia</i>	231 (11.8)	0.018 (0.0039)	0.046 (0.0129)	0.05 (0.119)	26.2	0.022	205
<i>N. stricta</i>	263 (12.7)	0.014 (0.0020)	0.021 (0.0065)	0.01 (0.075)	39.9	0.016	206
<i>A. vulneraria</i>	198 (4.3)	0.044 (0.0081)	0.056 (0.0267)	0.04 (0.117)	11.6	0.052	201
<i>G. verum</i>	192 (3.7)	0.042 (0.0053)	0.017 (0.0190)	0.10 (0.233)	15.1	0.044	192
<i>E. horridum</i>	208 (5.0)	0.025 (0.0025)	0.015 (0.0095)	0.05 (0.170)	25.0	0.026	191
June	222 (5.6)	0.031 (0.0041)	0.023 (0.0152)	0.09 (0.208)	15.6	0.042	214
July	226 (7.5)	0.023 (0.0037)	0.042 (0.0131)	0.04 (0.109)	21.8	0.028	210
August	208 (8.0)	0.021 (0.0038)	0.050 (0.0131)	0.04 (0.101)	22.6	0.026	191
September	192 (7.2)	0.022 (0.0042)	0.053 (0.0145)	0.05 (0.104)	20.9	0.028	180

† In brackets are standard errors of means for curve coefficients. A: total gas as the asymptote (ml/g DM); b, c: rate constants (per h and per h^{1/2}); T: lag time (h); $t_{1/2}$ and $\mu_{1/2}$: time (h) required to reach 0.50 total gas production and fractional degradation rate (per h) at that time.

among species regardless of the method used. However, *E. horridum* recorded the lowest digestibility when calculated from chemical composition ($P < 0.05$), but when this parameter was estimated from *in vitro* enzymatic digestion or gas production, it was no different to *N. stricta* or *F. eskia*. *A. vulneraria* and *G. verum* showed higher digestibility values than the other species ($P < 0.001$). Differences among collection dates in digestibility

were only detected with estimates from gas production, being highest in June, and higher in July than in September ($P < 0.001$).

The possible relationship between the different parameters studied was evaluated by a principal component analysis (Figure 3). Factor I explained 0.66 of total variance, and represents a gradient of quality, since parameters of cell wall composition, that are generally inversely related to forage quality

Table 4 Estimates of digestibility (g/kg dry matter) from chemical composition (DMDc), *in vitro* enzymatic digestion (DMD_e) and gas production (OMDg) for each species and each collection date

	DMDc	DMD _e	OMDg
<i>F. eskia</i>	545 ^a	541 ^b	487 ^b
<i>N. stricta</i>	524 ^a	462 ^b	472 ^b
<i>A. vulneraria</i>	620 ^a	727 ^a	568 ^a
<i>G. verum</i>	554 ^a	687 ^a	544 ^a
<i>E. horridum</i>	340 ^b	428 ^b	473 ^b
June	508	566	570 ^a
July	493	517	513 ^b
August	513	596	487 ^{bc}
September	552	596	465 ^c
Residual s. d.	51.9	55.5	23.5
Significance of:			
species effect	***	***	***
date effect			***

^{a,b,c} When comparing either species or dates of sampling, different superscripts indicate significant differences ($P < 0.05$).

Figure 3 Distribution of the different parameters used to compare the nutritive value according to factor I and II of the principal component analysis.

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appeared in the negative part of this axis whereas nitrogen content, gas production parameters and OMDg are placed in the positive part of the axis. The negative extreme of factor II was related to the lignin content, and the chemical digestibility estimation appeared in the positive part of the axis, whereas the enzymatic digestibility was related to both OMDg and gas production results. Together, both factors I and II explained 0.85 of total variance. Figure 4 shows a projection of species-collection-date cases of the early PCA axis. Samples of *A. vulneraria* (Ant) and *G. verum* (Gal) at all dates were located in the positive rank of factor I, indicating higher gas production and lower cell walls and lignin proportions, slightly higher in the June-July than in the August-September periods. June samples of *E. horridum*, (Ech), *F. eskia* (Fes) and *N. stricta* (Nar) also appear in the positive part of factor I, the former with a low factor II coefficient because of its high lignin proportion. For the grasses, it seems that their quality is more affected by their cell wall proportion than by microbial fermentation because they appear in the negative part of factor I.

Correlation coefficients among the studied parameters are shown in Table 5. Significant positive correlations have been observed between the volume of gas produced at 12, 24 or 48 h and OMDg, N content and enzymatic digestibility. These variables are also negatively correlated with NDF and ADF proportions, in agreement with the principal component analysis. However, the correlation coefficient between gas production and N content increased with the time of incubation, being higher at 48 than 24 h, and not significant at 12 h of fermentation.

Discussion

Both the volume of gas produced after 24 h of incubation (Menke and Steingass, 1988) and the parameters of the fitted curve (Khazaal *et al.*, 1995) have been used successfully to predict the apparent digestibility of forage foods, showing better results than other approximations using enzyme digestion or chemical composition (Aiple *et al.*, 1996; Minson, 1998). However, since fermentation of crude protein is inversely related to gas production, (Menke and Steingass, 1988; González Ronquillo *et al.*, 1998), it is necessary to consider the N content of foods for a better fit, as has been used to calculate average OMDg results in Table 4.

Digestibility estimates from the different methods were different ($P < 0.01$) with DMDe showing the highest values. Mean values of estimates from gas production compared with those from chemical composition did not differ significantly but values

from the two methods were not correlated, as they were with enzymatic and gas production estimates (Table 5). The position of DMDe, OMDg and DMDc in the PCA analysis indicates that the latter is the poorer estimator of digestibility compared with the others. In addition, digestibility estimation from gas production showed a lower coefficient of variation than both DMDc and DMDe (0.05, 0.10 and 0.10, respectively). The distance of DMDc from the rest of the quality factors in Figure 3 can be explained by the influence of lignin proportion in this digestibility estimator (Van Soest, 1994). *In vitro* enzymatic digestibility is a useful method for treatment comparison, although its results differ from *in vivo* apparent digestibility (Weiss, 1995). The fact that the equation used for OMDg estimation (Menke and Steingass, 1988) was established with volume readings in glass syringes, whereas in this study a pressure system was used, must be considered. However, we lack equations for estimating digestibility from gas pressure systems but the differences in our estimates were of sufficient magnitude as to probably not affect the ranking.

Differences in microbial digestion among the species studied were mainly based on the rate at which the cell contents and the pectin of the cell walls were fermented, since differences diminished with the time of incubation, and became not significant after 48 h of incubation (Figure 1). In this way, *A. vulneraria* and *G. verum*, with a higher proportion of cell contents, were the most fermentable species. In addition, the cell walls of legumes and other dicotyledonous species are rich in pectin, which are rapidly degraded by the rumen microbes (Hatfield and Weimer, 1995). In agreement with the low fermentation rates of grasses and *E. horridum* in the present study, López *et al.* (1991b) observed that among the species from a meadow at 1150 m above sea level, grasses have a slower rumen fractional degradation rate than legumes and other species. A higher rumen degradation rate of legumes compared with grasses has already been reported (Beever *et al.*, 1986; Grenet, 1989).

Despite the stage of maturity being more advanced for the legume (*A. vulneraria*) on nearly all collection dates, it produced a higher volume of gas than the herbaceous dicotyledonous variety *G. verum* for the first 8 h of incubation (Figure 1). Furthermore, the N content of *A. vulneraria* was not affected to a great extent by date, being reduced by a factor of only 0.06 from June to September. In contrast, that of *G. verum* was reduced by a factor of 0.41 in the same period. It is worth noting the reduction of the cell wall proportion, and the increase in enzymatic digestion in *A. vulneraria* as the summer advances, with no

apparent changes in gas production. This apparent contradiction has been observed previously in this species (García-González and Alvera, 1986) and also in other dicotyledenous species (Hanley and McKendrick, 1983). The high digestibility of *A. vulneraria* which remained constant throughout the vegetative period suggests that this species is potentially a good forage resource for animal production in poor soils (Often, 1998) or in mountain production systems. Among the other species, there were no major differences in microbial fermentation, as might have been expected from their NDF and ADF content.

In the current study, the date of sampling did not affect chemical composition, except for N proportion, nor gas production until 24 h incubation. The regrowth observed in some samples of *G. verum* and *E. horridum* in September seems not to have affected their chemical composition to a great extent. López *et al.* (1991a) even reported higher fibre contents and lower digestibility in mountain meadows harvested in June compared to subsequent cuts, probably because of a lower leaf to stem ratio. However, in our case from 24 h onwards there was a linear negative effect of the sampling date on gas production. Although the extent of lignification has been considered to be one of the main parameters affecting forage digestibility (Jung and Deetz, 1993), no significant correlation was observed with gas production at 12, 24 or 48 h (Table 5). Therefore, the effect of date on fermentation must be explained by differences in the anatomical structure of forages. In this sense, physical structures may form a barrier that prevents microbial action on potentially fermentable tissues in samples from August and September compared with June and July (Figure 2). In agreement with this hypothesis, the lignification of plants as maturity advances is not the only factor affecting microbial fermentation, but more importantly its location in specific tissues must be implicated (Wilson and Hatfield, 1997), especially with long fermentation periods. This may also explain why *in vitro* enzymatic digestibility agrees better with gas production results than chemical composition estimates with gas production results (Aiple *et al.*, 1996), and therefore both the former methods seem more accurate than chemical analyses for evaluating forages at different stages of maturity.

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(Received 15 June 2002—Accepted 5 December 2002)