

Diurnal variations on pasture chemical composition and fermentability

Variaciones diurnas en la composición química y la fermentescibilidad de pasturas

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Abstract

The aim of this work was to study diurnal variations in chemical composition and ruminal fermentability of a wide range of forages (*A. sativa*, *L. multiflorum*, *L. perenne*, *L. hybrid*, *B. auleticus*, *M. sativa*, *T. pratense*, *T. repens*, *T. alexandrinum*, *L. corniculatus*, *L. pedunculatus*, *L. tenuis*, *C. intybus*, *P. dilatatum*, *P. notatum*, *P. plicatum*, *P. lanceolata*, *S. sudanense*). Thirty paddocks involving different species at vegetative stage were sampled at 09:00, 13:00, and 17:00 h on the same day (treatments). Samples were subjected to chemical analysis and an in vitro gas production trial. Gas volumes were fitted to a dual-pool logistic model with single lag. A mixed model was used for statistical analysis, considering time of cut as fixed effect, and botanical group and season as random effects. The concentration of water-soluble carbohydrates (WSC) and the ratio WSC/N increased along the day, while neutral detergent fibre (NDF) and N contents decreased. This led to a shortening of lag time from the morning to the afternoon. Fibre content was associated with a longer lag time, a higher slow volume and lower fermentation rates. Pastures cut in the afternoon showed earlier onset of ruminal fermentation associated with lower concentrations of fibre and higher concentrations of soluble carbohydrates.

Keywords: carbohydrates, forage, in vitro gas production, photosynthesis, rumen fermentation.

Resumen

El objetivo de este trabajo fue estudiar las variaciones diurnas en la composición química y la fermentescibilidad ruminal en una amplia variedad de forrajes (*A. sativa*, *L. multiflorum*, *L. perenne*, *L. híbrido*, *B. auleticus*, *M. sativa*, *T. pratense*, *T. repens*, *T. alexandrinum*, *L. corniculatus*, *L. pedunculatus*, *L. tenuis*, *C. intybus*, *P. dilatatum*, *P. notatum*, *P. plicatum*, *P. lanceolata*, *S. sudanense*). Se muestrearon treinta parcelas de diferentes especies en estado vegetativo a las 09:00, 13:00 y 17:00 horas del mismo día (tratamientos). Las muestras fueron sometidas a análisis químicos y a una prueba de producción de gas in vitro. Los volúmenes de gas se ajustaron a un modelo logístico de dos compartimentos con una única fase de latencia. Para el análisis estadístico se utilizó un modelo mixto, considerando el momento de corte como efecto fijo y el grupo botánico y la estación como aleatorios. La concentración de azúcares solubles en agua y la relación azúcares solubles/N aumentaron a lo largo del día, mientras que los niveles de fibra en detergente neutro (NDF) y N disminuyeron. Esto provocó una disminución del tiempo de latencia de la mañana a la tarde. El contenido de fibra se asoció con una fase de latencia más larga, un mayor volumen de lenta producción y tasas de fermentación más lentas. Las pasturas cortadas en la tarde mostraron un inicio más temprano de la fermentación ruminal asociado a menor concentración de fibra y mayor concentración de azúcares solubles.

Palabras clave: carbohidratos, forraje, fotosíntesis, fermentación ruminal, producción de gas in vitro.

Introduction

In ruminant production systems of temperate regions, pastures are widely used as nutrient sources due to their low cost-benefit relationship. It is well known that N compounds of temperate pastures are rapidly degraded in the rumen (Fernandez-Turren *et al.*, 2020), but readily fermentable carbohydrates are often insufficient for N capture by ruminal bacteria, leading to a low N utilization and poor animal performance (Tebot *et al.*, 2012; Moorby and Fraser, 2021). A feature of forages is the accumulation of water-soluble carbohydrates (WSC) due to the photosynthetic process, leading to an increase in the content of readily fermented carbohydrates throughout the day (Kagan, 2022). Therefore, the diurnal increase of soluble carbohydrates could be used as a tool to increase the WSC/N ratio, and therefore, to improve rumen fermentation and N utilization in pastures of the rumen.

On the other hand, Rose *et al.* (2021) found that WSC content and dry matter (DM) digestibility are highly correlated, and that WSC content explains a large proportion of the variation in herbage intake. In this sense, Pozo *et al.* (2022) observed higher pasture intake and lower urinary N excretion in lactating cows fed with PMR (pasture + total mixed ration) when grazing was performed in the afternoon instead of in the morning. Also, Burns *et al.* (2005) reported higher DM and acid detergent fibre (ADF) digestibility of hays made from alfalfa cut in the afternoon compared to hays made from alfalfa cut in the morning. These observations are consistent with an improvement in ruminal fermentation of forages cut in the afternoon. In fact, Berthiaume *et al.* (2006), working with alfalfa, and Cajarville *et al.* (2015), working with alfalfa and fescue during autumn, observed that the WSC concentration and WSC/N ratio of fescue and alfalfa cut at 17:00 doubled the values observed at 09:00, and this led to an increase in the *in vitro* fermentation of these forages.

Another use of the increase in WSC along the day due to photosynthesis is ensiling. The preservation of feedstuffs as silage is a process that relies on the anaerobic fermentation of WSC with a concomitant accumulation of lactic acid (Chen *et al.*, 2023). In the case of low WSC feedstuffs, the addition of substances rich in soluble carbohydrates has been recommended, and some studies have revealed that the addition of WSC sources like molasses or cheese whey during the ensiling process increased the rate of *in vitro* gas production (Britos *et al.*, 2007) of pasture silages. This increase of WSC along the day could also be used as a tool for silage making, if this rise is enough to promote a rapid fermentation.

Although the natural increase of WSC along the day could be used for the aforementioned purposes, the available information about this phenomenon and its consequences on fermentation is still scarce

and restricted to few forage species (alfalfa and fescue). The aim of the present work was to study the variations in the chemical composition and its consequences on *in vitro* rumen fermentation along the day, using a wide range of pastures, collected in vegetative stage.

Materials and methods

This study followed the regulations of the ordinance “Use of Animals in University Experimentation, Teaching, and Research” of the Universidad de la República (2000) and was conducted according to the guidelines of the Animal Use Ethics Committee of the Veterinary Faculty in Montevideo (protocol CEUAFVET-472).

Sampling

Sampling was conducted from November 2004 to May 2005 on 30 commercial farms. Mean weather conditions of the sampling period (divided by seasons) are shown in Table 1. The soils of the farms corresponded to typic argiudolls (Soil Survey Staff, 2022) with clay loam texture and 2 to 5 % slopes. One plot per farm (established as pure stand) was selected for the experiment. The plots had different herbage mass (from 1000 to 4300 kg DM/ha, Table 2), but all were at vegetative stage, under grazing management, and corresponded to 18 species of 4 botanical groups: C3 grasses (n = 14), C4 grasses (n = 4), legumes (n = 10), and forbs (n = 2). Each plot was sampled the day before entering the animals to graze; the herbage mass was measured by cutting 20 quadrats of each forage (0.1 m²) to soil level with shears and sampled at three different times of the same day (09:00, 13:00, and 17:00 h), each time on 5 random places of the plot at 5 cm of the ground using manual shears. As the main objective was to study the changes in *in vitro* gas production of the forages throughout the day, the ‘treatment’ was the time of cut (09:00, 13:00, or 17:00 h), and the plots were used as replicates.

Table 1. Mean weather conditions by seasons along the sampling period

Season	Weather conditions					
	H (h) (¹)	AT (°C)	MT (°C)	MinT (°C)	Hum (%)	R (mm)
Spring (Sep 22 – Dec 21)	8.2	17.2	23.2	12.5	69.0	236.5
Summer (Dec 22 – Mar 21)	8.7	21.6	27.7	17.0	68.5	322.7
Autumn (March 22 – June 21)	5.5	14.5	19.4	10.6	80.4	286.4
Winter (June 22 – Sep 21)	5.3	10.9	15.1	7.10	80.2	253.0

Source: Instituto Nacional de Investigación Agropecuaria.

⁽¹⁾H: heliophany; AT: average temperature; MT: maximum temperature; MinT: minimum temperature; Hum: relative humidity; R: rainfall accumulated for the season.

Table 2. Species, sampling season, and herbage mass at the moment of sampling of the pastures used in the experiment

Species	Herbage mass (kg DM/ha)
Autumn	
<i>Avena sativa</i> (C3) ⁽¹⁾	4321
<i>Avena sativa</i> (C3)	1556
<i>Lolium multiflorum</i> (C3)	2915
<i>Lolium multiflorum</i> (C3)	1476
<i>Bromus auleticus</i> (C3)	1689
<i>Bromus auleticus</i> (C3)	1684
<i>Medicago sativa</i> (Leg)	4011
<i>Medicago sativa</i> (Leg)	2478
<i>Lotus corniculatus</i> (Leg)	1300
<i>Trifolium pratense</i> (Leg)	1304
<i>Trifolium pratense</i> (Leg)	2915
<i>Trifolium repens</i> (Leg)	1677
<i>Trifolium alexandrinum</i> (Leg)	2475
<i>Cichorium intybus</i> (forbs)	4011
Winter	
<i>Bromus auleticus</i> (C3)	1715
<i>Bromus auleticus</i> (C3)	1285
<i>Bromus auleticus</i> (C3)	1814
<i>Lolium multiflorum</i> (C3)	1960
<i>Lolium multiflorum</i> (C3)	2470
<i>Lolium perenne</i> (C3)	2010
<i>Lolium hybrid</i> (<i>multiflorum</i> * <i>perenne</i>) (C3)	2060
Spring	
<i>Avena sativa</i> (C3)	2475
<i>Paspalum dilatatum</i> (C4)	1780
<i>Paspalum notatum</i> (C4)	1193
<i>Paspalum plicatulum</i> (C4)	2213
<i>Lotus pedunculatus</i> (Leg)	1418
<i>Trifolium repens</i> (Leg)	1849
<i>Plantago lanceolata</i> (forbs)	1004
Summer	
<i>Sorghum sudanense</i> (C4)	2307
<i>Lotus tenuis</i> (Leg)	1764

⁽¹⁾C3: C3 grasses; C4: C4 grasses; Leg: legumes

Chemical analysis

Immediately after cutting, samples were frozen and stored at -20 °C. Before laboratory processing, they were oven-dried at 60 °C for 48 h and ground in a rotor mill with a 1 mm sieve (Fritsch GmbH, Idar-Oberstein, Germany). Dry matter (DM) was determined at 105 °C

to constant weight using fresh samples. Ash and crude protein (CP) were determined according to the methods of the Association of Official Analytical Chemists (2019) (942.05, and 2001.11, respectively); neutral detergent fibre (NDF) and acid detergent fibre (ADF) were assayed sequentially according to the methods proposed by Hall and Mertens (2023) using an ANKOM220 fibre analyzer (ANKOM Technology Corp., Macedon, NY, USA) with a heat-stable alpha-amylase, without sodium sulphite. The values expressed included residual ash. Organic matter (OM) was calculated as OM (g/kg) = 1 000 - Ash (g/kg). WSC were measured according to the method proposed by Sakiroglu *et al.* (2020).

In vitro gas production

For the in vitro gas production technique, 0.5 g of sample was used as substrate, and then incubated in 125 mL fermentation flasks. To each flask, 40.5 mL of N-free incubation medium (Williams *et al.*, 2005) was added, stoppered with butyl-rubber septa, and maintained at 4 °C for 8 h before inoculation to hydrate the substrate. Prior to inoculation, the flasks were warmed in a water bath at 39 °C, remaining there for the entire measurement period. Each flask was inoculated with 10 mL of rumen fluid and immediately stoppered with butyl-rubber septa and sealed with aluminium crimp. Rumen inoculum was collected from the ventral sac of the rumen of a lactating Holstein cow (550 kg LW), with rumen cannula, consuming a grass-legume pasture, corn silage, and grass silage (160 g CP/kg DM). All manipulations were performed under CO₂ flow. Gas pressure was measured using a digital pressure gauge (Cole-Parmer, Vernon Hills, IL, USA) connected to a syringe with a hypodermic needle at 2, 4, 6, 8, 10, 12, 18, 24, 48, 72, and 96 h after inoculation. Gas was vented after the pressure readings. The gas volume was estimated by the equation $V = 4.40 P + 0.09 P^2$ (V : gas volume in mL, and P : observed pressure in psi; $R^2 = 0.998$), obtained in a previous experiment under similar conditions. All in vitro gas production measurements were performed using duplicates, in two runs. The gas volume from each fermentation flask was fitted by nonlinear regression (PROC NLIN of SAS, version 8.02, SAS Institute Inc., Cary, NC, USA) according to the model:

$$V = \frac{VF}{1 + e^{[2 + 4 \times SRf \times (L - t)]}} + \frac{VS}{1 + e^{[2 + 4 \times SRf \times (L - t)']}}$$

Where V is the accumulated gas production at time (t), V_f is the final gas volume of fast production (fast volume, mL gas/g DM incubated), V_s is the final gas volume of slow production (slow volume, mL gas/g DM incubated), SR_f and SR_s are the specific gas production rates (h^{-1}), and L is the lag time (h) (Smith *et al.*, 2019).

Statistical analysis

Data analysis was performed using PROC MIXED of SAS (version 8.02, SAS Institute Inc., Cary, NC, USA) using the following model:

$$Y_{ijkl} = \mu + BG_i + S_j + H_k + e_{ijkl}$$

Where Y is the studied variable, μ is the overall mean, BG_i is the random effect of the botanical group ($i = C3$ grasses, $C4$ grasses, legumes or forbs), S_j is the random effect of the season ($j =$ spring, summer, autumn or winter), H_k is the effect of the time of cut ($k = 09:00, 13:00, \text{ or } 17:00$ h), and e_{ijkl} is the residual error. The plot was considered as the experimental unit and included as subject in the model. Comparisons between means for different times of cut were done by pre-planned orthogonal contrasts; morning cuts were compared with afternoon ones (09:00 vs. 13:00 + 17:00 h) and afternoon cuts were compared among them (13:00 vs. 17:00 h). Differences were considered statistically significant at $p < 0.05$ and trends at $0.05 < p < 0.10$.

Linear correlations (Pearson Correlation Coefficients, r) were estimated for chemical components and in vitro gas production means. The STEPWISE procedure of SAS was used to predict gas production parameters based on chemical composition using a backward selection, including variables in the model when significant at $p \leq 0.15$.

Results

No significant interactions between botanical groups (BG) and cutting schedule (CS) were detected for any chemical composition variables evaluated (Table 3). The WSC concentration and WSC/N ratio were lower when plants were cut in the morning (09:00 h).

The cutting time affected the SRf, Vs, and SRs, which were lower at 9:00 h. There was a significant interaction between the botanical group and the cutting schedule for SRs, only forbs showed differences among the different times of cut (0.016, 0.037, and 0.026 h^{-1} at 9, 13, and 17 h, respectively; $p < 0.001$).

Linear correlations between chemical composition and fermentation parameters are shown in Table 4. NDF and ADF contents were associated with a longer lag time, a higher slow volume (Vs), and a lower rate (mainly SRf). NDF with L and NDF with SRf were correlations higher than 0.6. A higher N concentration was related with a higher slow volume and rate (Vs and SRs). Meanwhile, higher WSC concentrations were associated with a higher slow volume (Vs), but with a lower fast volume and rate (Vf, SRf).

Table 5 shows the best equations predicting gas production according to the chemical composition of the forages. The coefficients of determination were higher than 60 % for L and Vs. For Vf and SRs, they were lower than 30 %. NDF or ADF were included in all equations with the highest relative weights, except for Vs, where WSC was the best single predictor.

Table 3. Effect of the cutting schedule (CS) on chemical composition and in vitro fermentation characteristics of forages

	Cutting Schedule (h)			SEM ⁽¹⁾	p-value		
	09:00	13:00	17:00		CS ⁽²⁾	9 vs 13 + 17 ⁽³⁾	13 vs 17
DM, g/kg ⁽⁴⁾	199.9	208.2	217.7	3.173	0.588	0.354	0.490
Composition, g/kg DM basis							
OM	888.9	889.8	890.7	0.99	0.526	0.288	0.451
NDF	461.7	444.9	434.4	8.337	0.016	0.005	0.142
ADF	257.5	247.9	242.9	2.948	0.168	0.062	0.431
WSC	76.0	98.6	114.2	1.241	<.001	<.001	<.001
N	28.8	27.8	26.0	0.413	<.001	0.001	0.001
WSC/N	3.19	4.17	5.08	0.981	<.001	<.001	<.001
In vitro fermentation							
L, h ⁽⁵⁾	1.57	1.30	1.16	0.623	0.009	0.002	0.165
Vf, ml/g DM	120.95	123.03	126.62	6.152	0.260	0.169	0.218
SRf, h ⁻¹	0.09	0.09	0.09	0.008	0.215	0.178	0.514
Vs, ml/g DM	71.92	75.65	77.14	9.275	0.152	0.053	0.522
SRs, h ⁻¹	0.02	0.02	0.02	0.001	0.875	0.723	0.658

⁽¹⁾SEM: standard error of the means. ⁽²⁾CS: cutting schedule. ⁽³⁾9 vs. 13 + 17 and 13 vs. 17: p-value of pre-planned orthogonal contrasts (cutting at 09:00 h vs. 13:00 h plus 17:00 h, and 13:00 h vs. 17:00 h, respectively) for times of cut. ⁽⁴⁾DM: dry matter; OM: organic matter; NDF: neutral detergent fibre; ADF: acid detergent fibre; WSC: water soluble carbohydrates; N: Nitrogen. ⁽⁵⁾L: lag time; Vf: gas volume of fast production; SRf: specific rate of fast production; Vs: gas volume of slow production; SRs: specific rate of slow production.

Table 4. Linear correlation coefficients between chemical components and in vitro fermentation parameters for the forages (n = 85)

	L ⁽²⁾	Vf	SRf	Vs	SRs
DM ⁽¹⁾	-0.124 ⁽³⁾ (0.439) ⁽⁴⁾	-0.222 (0.164)	-0.323 (0.039)	0.194 (0.225)	0.006 (0.966)
OM	-0.395 (0.011)	0.085 (0.597)	0.060 (0.708)	-0.059 (0.711)	0.052 (0.745)
NDF	0.621 (<0.001)	-0.178 (0.266)	-0.658 (<0.001)	0.498 (0.009)	-0.357 (0.022)
ADF	0.575 (<0.001)	-0.385 (0.013)	-0.502 (<0.001)	0.530 (<0.001)	-0.160 (0.317)
N	-0.233 (0.143)	0.134 (0.403)	-0.025 (0.877)	0.480 (0.002)	0.340 (0.029)
WSC	0.145 (0.366)	-0.315 (0.045)	-0.359 (0.021)	0.729 (<0.001)	0.110 (0.450)

⁽¹⁾DM: dry matter; OM: organic matter; NDF: neutral detergent fibre; ADF: acid detergent fibre; WSC: water soluble carbohydrates. ⁽²⁾L: lag time (h); Vf: gas volume of fast production (ml gas/g DM incubated); SRf (h⁻¹): specific rate of fast production; Vs: gas volume of slow production (ml gas/g DM incubated); SRs (h⁻¹): specific rate of slow production. ⁽³⁾Linear correlation coefficient (r). ⁽⁴⁾Statistical probability (p).

Lag time was higher as NDF and ADF increased. Vf decreased with higher ADF proportions in forages but raised with NDF. The fast rate of gas production (SRf) decreased with the increase of NDF, which predicted more than 40 % of the variation. The slow rate of gas production (SRs) was reduced as NDF and N increased, but these fractions explained only 21 % of the variation.

Discussion

This study addresses the daily behaviour of chemical composition and its impact on the fermentability of forages. While the systematic sampling scheme used for all paddocks provides strength for studying the effect of time of cutting on all the variables, the fact that each sample was collected from independent paddocks in only one day per paddock, limits the inference on particular species. It is noteworthy that chemical composition changed in a similar manner for all forages throughout the day. The WSC concentration increased considerably during this time, being the main change in chemical composition (50 % higher at 17:00 h than at 09:00 h). At the same time, N concentration decreased, leading to a 59 % increase of the WSC/N ratio during the same period, higher than the WSC increase. These facts were already described by other authors for several species and seasons (Pozo *et al.*, 2022; Cajarville *et al.*, 2015; Delagarde *et al.*, 2000). Concurrently to the N decrease, NDF levels were lower at the afternoon cuts. These variations, due to a dilution effect, have been previously reported by Huntington and Burns (2008) for switchgrass, and Cajarville *et al.* (2015) for

Table 5. Linear regression analysis to predict in vitro gas production parameters from chemical composition of forages

Parameter	Equation	RSD ⁽³⁾	R ²	p
L ⁽¹⁾	-1.36 + 0.06 NDF ⁽²⁾	0.963	0.386	<0.001
	-0.87 + 0.07 NDF - 0.04 DM	0.897	0.481	<0.001
	-1.80 + 0.03 NDF - 0.06 DM + 0.11 ADF	0.841	0.555	<0.001
Vf	14.85 + 0.02 NDF - 0.04 DM + 0.14 ADF - 0.19 OM	0.804	0.605	<0.001
	160.10 - 1.53 ADF	19.74	0.148	0.013
SRf	161.65 - 2.69 ADF + 0.64 NDF	19.43	0.195	0.016
	-135.94 - 3.50 ADF + 1.03 NDF + 3.37 OM	18.91	0.258	0.011
SRs	0.141 - 0.001 NDF	0.017	0.433	<0.001
Vs	52.70 + 6.43 WSC	13.45	0.531	<0.001
	34.23 + 5.61 WSC + 0.49 NDF	12.33	0.616	0.006
SRs	0.0277 - 0.0002 NDF	0.006	0.128	0.022
	0.0218 - 0.0002 NDF + 0.0005 N	0.006	0.208	0.012

⁽¹⁾L: lag time (h); Vf: gas volume of fast production (ml gas/g DM incubated); SRf (h⁻¹): specific rate of fast production; Vs: gas volume of slow production (ml gas/g DM incubated); SRs (h⁻¹): specific rate of slow production. ⁽²⁾DM: dry matter; OM: organic matter; NDF: neutral detergent fibre; ADF: acid detergent fibre; WSC: water soluble carbohydrates. ⁽³⁾RSD: residual standard deviation; R²: coefficient of determination; p: statistical probability.

alfalfa and fescue. Nevertheless, these decreases were less important compared with the WSC increase, since NDF decreased 6 % and N diminished 10 %.

The influence of the cutting schedule of forage on ruminal fermentation has been previously reported, although restricted to few forage species. In this sense, Berthiaume *et al.* (2006) and Cajarville *et al.* (2015) observed an increase of gas volumes and gas production rates measured in vitro in alfalfa, and in alfalfa and fescue, respectively. However, the model used by Cajarville *et al.* (2015) had only a fractional rate value preventing to discriminate when the fermentation was more affected; in the present study, the model used to describe in vitro fermentation kinetics allows the separation of fast and slow fermentation fractions, while the use of contrasts provides information about when the fermentation was more affected.

An expected general enhance of the ruminal fermentation was not observed, and lag time (L) was the only fermentation parameter affected. This variable is related to the removal of degradation inhibitors and microorganism attachment to the substrate (Esen, 2023; Huhtanen *et al.*, 2008). Its shortening was significant only from 09:00 h to 13:00 h. Despite the increase of the highly fermentable substrate (WSC) in the afternoon, fast volume of gas (Vf) and its production rate were similar throughout the day. This was unexpected since these fermentation parameters have been related with the abundance of readily fermentable carbohydrates in the forages (Huntington and Burns, 2008).

The correlation analysis is consistent with the observation described above. Fibre content was associated with a longer lag time, a higher slow volume (Vs), and a lower rate (mainly SRf). A higher N concentration was related with a higher slow volume and rate (Vs and SRs). Meanwhile, a higher WSC concentration was associated with a higher slow volume (Vs), but with lower fast volume and rate (Vf, SRf), which can indicate the importance of readily fermentable carbohydrates throughout the entire fermentation process, rather than solely during early fermentation stages. In fact, in the afternoon, the whole fermentation process (Vf + Vs) produced on average 11 mL more gas per gram of dry matter incubated in the afternoon respect to the morning (the mean for all the forages studied was 193 mL/g DM, and 204 mL/DM for 09:00 h, and 17:00 h, respectively). This increase in the produced gas volume is desirable, as a greater magnitude of substrate fermentation implies higher nutrient uptake for the animal. Although in the present experiment the methane concentration in the produced gas was not measured, Dini *et al.* (2017) observed that low-fibre forages (also with a high proportion of non-fibre carbohydrates) produced less methane per unit of DM ingested compared with forages higher in fibre. Despite a consistent increase of WSC throughout the day in the forages, the weak relationship between WSC and the fermentation parameters prevent us to conclude that a high content of soluble carbohydrates ensures high rumen fermentation of forages. Both NDF and ADF led to a lower fermentation rate, delaying the fermentation process, but enhanced the slowly produced gas.

The regression equations remarked the importance of the amount and the type of fiber fractions, as NDF and ADF determined the ruminal fermentation kinetics, delaying the fermentation process. The fact that ADF depressed and NDF enhanced the rapidly produced gas is not strange, as it has been proposed that NDF could comprise fast- and slow-digestible fractions (Coblentz *et al.*, 2018). In addition, slowly produced gas (Vs) raised as NDF increased; but the WSC content was the main parameter of the prediction, explaining more than 50 % of the Vs variation. The relationship between NDF and WSC content may seem unusual, as the latter increased the slowly produced gas (Vs) more than NDF did. However, it is necessary to consider that higher NDF digestibility has been reported when diet proportions of molasses or sucrose increased (Torres *et al.*, 2021). On the other hand, WSC concentration and gas production rates were higher in the afternoon cuts, suggesting a relationship between them that could not be found or at least quantified in this experiment. Nevertheless, gas production was poorly predicted from chemical composition, since only for L and Vs the coefficient of determination (R^2) was higher than 0.6.

Conclusions

Despite the weak association between the increase in soluble sugars and the parameters indicating the speed and magnitude of ruminal fermentation, the observations of a shorter lag time and greater gas production throughout the fermentation process would indicate a more rapid onset of ruminal fermentation with persistent effects. These facts should be considered for grazing management and forage conservation. Still, more research is needed to study the influence of WSC on ruminal fermentative behaviour.

References

- AOAC. (2019). *Official Methods of Analysis*, 21st ed. Latimer, G. W. (Ed.). Gaithersburg: Association of Official Analytical Chemists International (AOAC).
- Berthiaume, R.; Tremblay, G.; Castonguay, Y.; Bertrand, A.; Bélanger, G.; Lafrenière, C. and Michaud, R. (2006). Length of the daylight period before cutting improves rumen fermentation of alfalfa assessed by *in vitro* gas production. *Journal of Animal Science*, 84(1), 102. <https://www.jtmtg.org/JAM/2006/abstracts/100.pdf>
- Britos, A.; Repetto, J. L.; Garcarena, D. and Cajarville, C. (2007). Efecto del suero de queso como aditivo de ensilajes de pastura sobre la conservación, los azúcares solubles y la producción de gas *in vitro*. *Agrociencia*, 11(2), 72-77. https://www.academia.edu/79438584/Efecto_del_suero_de_queso_como_aditivo_de_ensilajes_de_pastura_sobre_la_conservaci%C3%B3n_los_az%C3%BAcares_solubles_y_la_producci%C3%B3n_de_gas_in_vitro
- Burns, J. C.; Mayland, H. F. and Fisher D. S. (2005). Dry matter intake and digestion of alfalfa harvested at sunset and sunrise. *Journal of Animal Science*, 83(1), 262-270. <https://doi.org/10.2527/2005.831262x>
- Cajarville, C.; Britos, A.; Errandonea, N.; Gutiérrez, L.; Cozzolino, D. and Repetto J. L. (2015). Diurnal changes in water-soluble carbohydrate concentration in lucerne and tall fescue in autumn and the effects on *in vitro* fermentation. *New Zealand Journal of Agricultural Research*, 58(3), 281-291. <https://doi.org/10.1080/00288233.2015.1018391>
- Chen, L.; Wang, Y.; Li, X.; MacAdam J. W. and Zhang, Y. (2023). Interaction between plants and epiphytic lactic acid bacteria that affect plant silage fermentation. *Frontiers in Microbiology*, 14, 1164904. <https://doi.org/10.3389/fmicb.2023.1164904>
- Coblentz, W. K.; Akins, M. S.; Kalscheur, K. F.; Brink, G. E. and Cavadini, J. S. (2018). Effects of growth stage and growing degree day accumulations on triticale forages: 2. *In vitro* disappearance of neutral detergent fiber. *Journal of Dairy Science*, 101(10), 8986-9003. <https://doi.org/10.3168/jds.2018-14867>
- Delagarde, R.; Peyraud, J. L.; Delaby, L. and Faverdin, P. (2000). Vertical distribution of biomass, chemical composition and pepsin-cellulase digestibility in a perennial ryegrass sward: Interaction with month of year, regrowth age and time of day. *Animal Feed Science and Technology*, 84(1-2), 49-68. [https://doi.org/10.1016/S0377-8401\(00\)00114-0](https://doi.org/10.1016/S0377-8401(00)00114-0)
- Dini, Y.; Gere, J. I.; Cajarville, C. and Ciganda, V. S. (2017). Using highly nutritious pastures to mitigate enteric methane emissions from cattle grazing systems in South America.

- Animal Production Science*, 58(12), 2329-2334. <https://doi.org/10.1071/AN16803>
- Esen, S. (2023). Optimizing ruminant nutrition: Insights from a comprehensive analysis of silage composition and *in vitro* gas production dynamics using nonlinear models. *Biosystems*, 234, 105062. <https://doi.org/10.1016/j.biosystems.2023.105062>
- Fernández-Turren, G.; Repetto, J. L.; Arroyo, J. M.; Pérez-Ruchel, A. and Cajarville, C. (2020) Lamb fattening under intensive pasture-based systems: a review. *Animals*, 10(3), 382. <https://doi.org/10.3390/ani10030382>
- Hall, M. B. and Mertens, D. R. (2023). Comparison of alternative neutral detergent fiber methods to the AOAC definitive method. *Journal of Dairy Science*, 106(8), 5364-5378. <https://doi.org/10.3168/jds.2022-22847>
- Huhtanen, P.; Seppälä, A.; Ahvenjärvi, S. and Rinne, M. (2008). Prediction of *in vivo* neutral detergent fiber digestibility and digestion rate of potentially digestible neutral detergent fiber: Comparison of models. *Journal of Animal Science*, 86(10), 2657-2669. <https://doi.org/10.2527/jas.2008-0894>
- Huntington, G. B. and Burns, J. C. (2008). The interaction of harvesting time of day of switchgrass hay and ruminal degradability of supplemental protein offered to beef steers. *Journal of Animal Science*, 86(1), 159-166. <https://doi.org/10.2527/jas.2006-701>
- Instituto Nacional de Investigación Agropecuaria. (2023). Banco de datos agroclimático. Montevideo, Uruguay. <http://www.inia.uy/gras/Clima/Banco-datos-agroclimatico>
- Kagan, I. A. (2022). Water- and ethanol-soluble carbohydrates of temperate grass pastures: a review of factors affecting concentration and composition. *Journal of Equine Veterinary Science*, 110, 103866. <https://doi.org/10.1016/j.jevs.2022.103866>
- Moorby, J. M. and Fraser, M. D. (2021). Review: New feeds and new feeding systems in intensive and semi-intensive forage-fed ruminant livestock systems. *Animal*, 15(S1), 100297. <https://doi.org/10.1016/j.animal.2021.100297>
- Pozo, C. A.; Kozloski, G. V.; Cuffia, M.; Repetto, J. L. and Cajarville, C. (2022). Changing the grazing session from morning to afternoon or including tannins in the diet was effective in decreasing the urinary nitrogen of dairy cows fed a total mixed ration and herbage. *Journal of Dairy Science*, 105(6), 4987-5003. <https://doi.org/10.3168/jds.2021-21149>
- Rose, M. F.; Waldron, B. L.; Isom, S. C.; Peel, M. D.; Thornton, K. J.; Miller, R. L.; Rood, K. A.; Hadfield, J. A.; Long, J.; Henderson, B. and Creech, J. E. (2021). The effects of organic grass and grass-birdsfoot trefoil pastures on Jersey heifer development: Herbage characteristics affecting intake. *Journal of Dairy Science*, 104(10), 10879-10895. <https://doi.org/10.3168/jds.2020-19563>
- Sakiroglu, M.; Dong, C.; Hall, M. B.; Jungers, J. and Picasso, V. (2020). How does nitrogen and forage harvest affect belowground biomass and nonstructural carbohydrates in dual-use Kernza intermediate wheatgrass? *Crop Science*, 60(5), 2562-2573. <https://doi.org/10.1002/csc2.20239>
- Smith, W. B.; Miller, M. D.; Crossland, W. L.; Callaway, T. R.; Tedeschi, L. O. and Rouquette Jr., F. M. (2019). *In vitro* gas production including methane from bermudagrasses supplemented with dried distillers grains with solubles. *Applied Animal Science*, 36(2), 172-182. <https://doi.org/10.15232/aas.2019-01916>
- Soil Survey Staff. (2022). *Keys to soil taxonomy - 13th edition*. United States Department of Agriculture, Natural Resources Conservation Service. <https://nrcspad.sc.egov.usda.gov/DistributionCenter/product.aspx?ProductID=1709#:~:text=Natural%20Resources%20Conservation%20Service&text=Keys%20to%20Soil%20Taxonomy%2D13th%20Edition%20provides%20the%20taxonomic%20keys,is%20available%20in%20PDF%20format>
- Tebot, I.; Cajarville, C.; Repetto, J. L. and Cirio, A. (2012). Supplementation with non-fibrous carbohydrates reduced fiber digestibility and did not improve microbial protein synthesis in sheep fed fresh forage of two nutritive values. *Animal*, 6(4), 617-623. <https://doi.org/10.1017/S1751731111002011>
- Torres, R. de N. S.; Bertoco, J. P. A.; de Arruda, M. C. G.; Coelho, L. M.; Paschoaloto, J. R.; Júnior, G. A. A.; Ezequiel, J. M. B. and Almeida, M. T. C. (2021). Meta-analysis to evaluate the effect of including molasses in the diet for dairy cows on performance, milk fat synthesis and milk fatty acid. *Livestock Science*, 250, 104551. <https://doi.org/10.1016/j.livsci.2021.104551>
- Williams, B. A.; Bosch, M. W.; Boer, H.; Verstegen, M. W. A. and Tamminga, S. (2005). An *in vitro* batch culture method to assess potential fermentability of feed ingredients for monogastric diets. *Animal Feed Science and Technology*, 123-124(P1), 445-462. <https://doi.org/10.1016/j.anifeedsci.2005.04.031>