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Original article

Effect of wilting and ensiling on ruminal degradability of temperate grass and legume mixtures

José Luis REPETTO, Cecilia CAJARVILLE*, Jorge D'ALESSANDRO, Ana CURBELO, Claudio SOTO, Daniel GARÍN

Departamento de Nutrición Animal, Facultad de Veterinaria, Universidad de la República, A. Lasplaces 1550, 11600 Montevideo, Uruguay

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Abstract – Changes in chemical nitrogen fractions and ruminal degradability of wilted and ensiled temperate forages were studied. Seven cultivated pastures from Uruguayan farms were sampled for their study in each of the following silage-making steps: fresh forage (F), wilted forage (W), and silage (S). Samples were analysed for crude protein (CP), soluble protein in buffer phosphate (SP), and neutral and acid detergent insoluble nitrogen (NDIN and ADIN). The degradability of dry matter (DM) and nitrogen (N) were measured in situ in three cows, and the effective degradability (ED) was calculated using outflow rumen rates of 3 and 6% per h (ED03 and ED06). Wilting reduced both ED06 of DM (F = 56.29%, W = 54.25%; $P = 0.033$) and N (F = 71.65%, W = 68.03%; $P < 0.001$). However, wilting did not produce significant changes in the chemical fractions. Forage fermentation produced an increase in SP (Average value of fresh and wilted forages (F + W) = 27.35%, S = 44.25% of CP; $P < 0.001$) and ADIN (F + W = 9.90%, S = 13.56% of CP; $P = 0.014$). Ensiling increased the soluble fraction (a) (F + W = 43.15%, S = 59.68%; $P < 0.001$), the undegradable fraction (u) (F + W = 16.52%, S = 18.45%; $P = 0.007$) and decreased the non-soluble degradable fraction (b) (F + W = 40.34%, S = 21.87%; $P < 0.001$) of N. Thus, fermentation produced significant changes in the chemical N fractions and ruminal degradability of DM and N in temperate regions, while wilting affected only the latter.

forage / wilting / fermentation / silage / rumen degradability

Résumé – **Effect du préfanage et de l'ensilage sur la dégradabilité ruminale des fourrages de prairies tempérées.** Les variations des paramètres de la dégradation ruminale de la matière sèche (DM) et de l'azote ont été étudiées sur le fourrage vert sur pied (F), préfané (W) et ensilé (S). Ces fourrages provenaient de sept prairies tempérées en Uruguay. Les teneurs en matières azotées totales (CP), en azote soluble (SP) et en azote lié aux parois végétales (NDIN et ADIN) ont été déterminées. Les dégradabilités ruminales de la DM et de l'azote (N) ont été déterminées par la méthode in situ sur 3 vaches et la dégradabilité théorique (ED) a été calculée en utilisant un taux de sortie des particules du rumen, respectivement, de 3 et 6 % par h (ED03 et ED06). Le préfanage a entraîné une

* Corresponding author: nutrag@adinet.com.uy

diminution de la dégradabilité de la DM (ED06, F = 56,3 % vs. W = 54,3 %; P = 0,033) et de l'azote (F = 71,7 % vs. W = 68,0 %; P < 0,001) mais n'a pas entraîné de changements importants des paramètres chimiques. La fermentation des fourrages a produit une augmentation de la teneur en azote soluble (F + W = 27,4 % vs. S = 44,3 % de CP; P < 0,001) et de l'ADIN (F + W = 9,9 % vs. S = 13,6 % de CP; P = 0,014). Elle a eu comme conséquence une augmentation de la fraction soluble (a) (F + W = 43,2 % vs. S = 59,7 %; P < 0,001) et de la fraction non dégradable (u) (F + W = 16,5 % vs. S = 18,5 %; P = 0,007) et une diminution de la fraction dégradable non soluble (b) (F + W = 40,3 % vs. S = 21,9 %; P < 0,001). L'ensilage a entraîné des changements des paramètres de la dégradation ruminale de la matière sèche et de l'azote tandis que le préfanage a seulement affecté la dégradabilité de l'azote.

fourrage / préfanage / fermentation/ensilage / dégradabilité ruminale

1. INTRODUCTION

Plant respiration, enzymatic activities of the plant and homo- and hetero-fermentative microorganisms have been indicated as being responsible for carbohydrate oxidation, protein degradation, and amino acid deamination during wilting and ensiling [8, 9, 19]. A consequence of these processes may be a reduction in the nutritive value of silage compared with standing fresh forages [6, 20].

Spring excess temperate grass-legume pastures are usually preserved as silage or hay by Uruguayan dairy farms. Making silage from this type of forage always requires a wilting period to reduce the water content of the forage and to increase the relative content of soluble carbohydrates [9]. Under experimental conditions, high quality silages have been obtained from Uruguayan temperate pastures [7]. However, D'Alessandro et al. [5] carried out field trials in this country and observed that the nutritive value of farm silages were low as a consequence of the advanced maturity of forages and inadequate fermentation during silage-making steps. According to these authors, the inadequate fermentation was evidenced by high pH and ADIN values.

The purpose of this work was to study the changes in nitrogen fractions and ruminal degradability characteristics of the forage during the wilting and fermentation-storage periods of silage making in Uruguayan dairy farms.

2. MATERIALS AND METHODS

2.1. Forage samples

Seven cultivated pastures composed of mixtures of grasses (*Festuca arundinacea, Lolium multiflorum*) and legumes (*Trifolium repens, Trifolium pratense, Medicago sativa*) from commercial farms in the dairy region next to Canelones City, Uruguay (34° S and 55° W) were used. Each pasture was sampled in three steps of the silage-making process: F, fresh forage; W, wilted forage; and S, silage. Fresh and wilted forages were sampled in the spring of 1997 (October 22–November 1). Silages were sampled between February 15 and March 3 of 1998.

Each sample of F was collected immediately after cutting at ten different sites of the pasture. Botanical composition of the canopy was also estimated from ten samples. Forage was cut at 2.5 cm height in an area of 0.01 m^2 (0.1 × 0.1 m) and the sampled forage was separated in legumes, grasses, others, and dried residues (Tab. I). The W forage was sampled after a field-wilting period (7.8 ± 1.2 h) that was determined by the farmer according to weather conditions. Wilted forage was baled in round bales of approximately 700 kg, wrapped and stored outdoors. Each sample of S was collected from 10 different bales of each pasture. A total of 21 samples were collected (F n = 7; W n = 7 and S n = 7). A sub-sample of each one was reserved for the determination of DM at 105 °C. All forage samples were freeze-dried and ground to

Table I. Botanical and chemical composition of each pasture used in the trial.

Botanical composition[a]	kg DM·ha^{-1}	DM (g·kg^{-1} FM)	Chemical composition (g·kg^{-1} DM)			
			OM	CP	ADF	NDF
(1) L: 46.7, G: 44.0, O: 2.40, R: 7.10 *(Trifolium repens, Festuca arundinacea)*	6094	150.8	892.6	124.4	402.6	549.7
(2) L: 45.7, G: 37.9, O: 2.4, R: 14.0 *(Medicago sativa, Lolium multiflorum)*	7057	194.0	928.3	134.8	359.8	478.1
(3) L: 48.2, G: 39.7, R: 12.1 *(Trifolium pratense, Lolium multiflorum)*	8626	168.6	910.0	101.5	383.7	512.3
(4) L: 62.5, G: 24.0, O: 4.4, R: 9.1 *(Medicago sativa, Lolium multiflorum)*	4165	247.5	928.6	140.1	369.7	509.9
(5) L: 67.6, G: 27.3, R: 5.1 *(Trifolium pratense, Lolium multiflorum)*	6759	168.1	928.8	116.6	356.4	428.3
(6) L: 45.0, G: 26.3, O: 17.3, R: 11.1 *(Trifolium pratense, Lolium multiflorum)*	7900	224.5	927.9	109.8	377.4	496.8
(7) L: 25.6, G: 55.6, O: 11.5, R: 7.30 *(Medicago sativa, Lolium multiflorum)*	5946	217.0	921.4	94.45	351.3	493.4

DM = dry matter; FM = fresh matter; OM = organic matter; CP = crude protein; ADF = acid detergent fibre; NDF = neutral detergent fibre.
[a] Percentage of legumes (L), grasses (G), others (O), and dried residues (R) of each pasture.

pass a 2-mm screen for use in degradability trials, and to pass a 1-mm screen for chemical analysis.

2.2. Chemical analysis

All samples (10 repetitions for each forage) were analysed for dry matter (DM), organic matter (OM), crude protein CP [2], neutral detergent fibre (NDF) and acid detergent fibre (ADF) [17]. Forages were also analysed for soluble protein (SP) in buffer phosphate [12], neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) by Kjeldahl analysis of NDF and ADF residues, respectively. The contents of SP were expressed as a percentage of CP. NDIN and ADIN contents were expressed as a percentage of total N.

2.3. Rumen degradability

Three Holstein heifers (non-pregnant) fitted with rumen cannula, weighing 490 kg BW on average, were used to perform in situ incubations. Cows were fed 2/3 forage (50% grass silage and 50% grass-legume hay) and 1/3 concentrate diet. The whole diet contained 122.0 g CP·kg^{-1} DM and 314.0 g ADF·kg^{-1} DM. The ration was distributed daily at an intake level of 70 g DM·kg BW$^{-0.75}$ in two equal meals at 8 and 19 hours.

The samples were incubated in nylon bags (10 × 20 cm; 52 µm pore size, ANKOM Technology Corp., Fairport, NY, USA), using 5 g of ground material, for 2, 4, 8, 12, 24, 48 and 72 h. In order to obtain two bags per animal and incubation time, two series of incubations for all forage samples were conducted within each animal on different days. After being removed from the rumen, the bags were washed with tap water (15 °C) during 5 minutes and were frozen afterwards. Once defrosted, the bags were manually washed for 10 minutes with running tap water (15 °C), dried at 80 °C for 48 h and analysed for DM and N contents. Three

Table II. Chemical composition (g·kg^{-1} DM) of temperate grass-legume forages at different steps of silage making.

Item		Treatment			P^a	
	F	W	S	SEM	F vs. W	(F+W) vs. S
DM (g·kg^{-1} FM)	195.8	372.4	380.6	1.423	<0.001	<0.001
OM	919.7	918.3	910.4	0.228	0.682	0.010
CP	117.4	115.2	115.1	3.620	0.678	0.797
NDF	495.5	515.5	555.3	1.339	0.312	0.010
ADF	371.6	387.7	434.5	0.928	0.242	<0.001
pH mean			4.49			
min			4.43			
max			5.90			

DM = dry matter; FM = fresh matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; F = fresh forage; W = wilted forage; S = ensiled forage; SEM = standard error of the mean.
[a] Probability of the orthogonal contrast.

additional bags of each sample (n = 21) were reserved for zero incubation (time 0 h) that involved the washing procedure without rumen incubation. Disappearance of DM and N were fitted by non-linear regression for each sample and cow to the exponential model proposed by Ørskov and McDonald [15]:

$$d\,(\%) = a + b\,(1 - e^{-k_d t})$$

where d is the percentage of material in the bag that disappeared at time t, a (%) is the soluble fraction, b (%) is the non-soluble but degradable fraction and k_d (% per h) is the fractional degradation rate of fraction b. The u (%) is the undegradable fraction and it was calculated as 100 – (a + b). The effective degradability (ED) (%) was estimated as:

$$ED = a + (b\,k_d) / (k_d + k_p)$$

using constant particulate outflow rates through the rumen (k_p) of 3% per h (ED03) as a low flow and 6% per h (ED06) as a rapid flow.

2.4. Statistical analysis

The effect of treatments (F, W and S) on chemical composition (DM, OM, CP, NDF, ADF, SP, NDIN, ADIN and NDIN-ADIN) was analysed by analysis of variance considering the pasture (n = 7) effect. The effect of treatments on degradability were analysed by analysis of variance considering pasture (n = 7) and animal (n = 3) effects. The means were compared using orthogonal contrasts. Linear correlation analysis between chemical and degradation parameters were made.

3. RESULTS

3.1. Chemical composition and nitrogen fractions

Wilting did not change the chemical composition of the forage (Tab. II). After fermentation and 60 days of storage, the CP contents were not changed but a significant reduction in the OM indicated losses of volatile products. The NDF and ADF were affected by fermentation, and they increased 12.1% and 16.9%, from the F to S forage, respectively. Chemical N fractions were not affected by wilting (Tab. III). However, fermentation and storage increased the contents of SP (F+W = 27.35% vs. S = 44.25%; $P < 0.001$) and ADIN (F+W = 9.90% vs. S = 13.56%; $P < 0.014$).

Table III. Effects of wilting and ensiling on chemical nitrogen fractions of temperate grass-legume forages.

	Treatment				P^a	
Item	F	W	S	SEM	F vs. W	(F+W) vs. S
CP ($g \cdot kg^{-1}$ DM)	117.4	115.2	115.1	3.620	0.678	0.797
SP	27.3	27.4	44.3	2.905	0.987	<0.001
NDIN	17.1	20.4	20.6	1.829	0.232	0.423
ADIN	9.49	10.3	13.6	1.033	0.586	0.014
(NDIN–ADIN)	7.6	10.1	7.07	0.928	0.087	0.140

CP = crude protein; SP = soluble protein; NDIN = neutral detergent insoluble nitrogen; ADIN = acid detergent insoluble nitrogen; F = fresh forage; W = wilted forage; S = ensiled forage; SEM = standard error of the mean. SP expressed as the percentage of CP; NDIN, ADIN and NDIN–ADIN expressed as the percentage of total N.
[a] Probability of the orthogonal contrast.

3.2. Rumen degradability

Degradation of DM and N were affected by treatments throughout the silage–making steps (Tab. IV).

Both wilting (measured by the contrast F vs. W) and fermentation and storage (measured by the contrast F+W vs. S) promoted a significant decrease in DM degradability. DM effective degradability calculated with a rapid rate of passage (ED06) was the highest for F (56.29%), followed by W (54.25%) and by S (50.88%). When a slower rate of passage was applied (ED03), a similar trend was observed but with no differences between F and W (P = 0.092). Wilting led to a diminution in the *a* fraction (–3.67 points) and an increase in the *b* fraction (+3.91 points), with no changes in the k_d and the *u* fraction. Meanwhile, the fermentation and storage processes led to a reduction of nearly 2 points in the k_d, and only this parameter was significantly affected.

Degradation of nitrogen compounds varied according to the step of the process (Tab. IV). In the first step, wilting led to a significant reduction of 3.62 points in the ED06 (F = 71.65, W = 68.03; $P < 0.001$) and 2.59 points in the ED03 (F = 76.5, W = 73.91; $P = 0.002$). In the second step, fermentation and storage generated an increase in ED03 and ED06. However, only ED06 was significantly affected (F+W = 68.34% vs. S = 73.45%, $P < 0.001$) whereas ED03 had a tendency to increase (F+W = 75.21% vs. S = 76.49%, $P = 0.064$). During wilting an increase in the *b* fraction of 4.10 points was recorded, with a lower degradation rate (k_d) (–2.22 points). After fermentation and storage, the *a* fraction augmented more than 16 points, the *b* fraction decreased nearly 18.5 points (F+W = 40.34% vs. S = 21.87%, $P < 0.001$), and the *u* fraction increased slightly (F+W = 16.52% vs. S = 18.45%, $P = 0.007$).

4. DISCUSSION

The variability observed between forages was expected since they arose from different farms and pastures. The CP content of the grass-legume temperate pastures was low, and NDF and ADF contents indicated an advanced stage of maturity (Tab. II). These values were consistent with the type of forage usually used to make silage by Uruguayan farmers [5]. On average, 27.31% of lyophilised fresh forage CP was soluble in phosphate buffer (SP), and this value was lower than the 35% presented by Jarrige et al. [11] for temperate forages, especially

Table IV. Effects of wilting and ensiling on rumen degradation characteristics of temperate grass-legume forages.

Item	Treatment			SEM	P^{a}	
	F	W	S		F vs. W	(F+W) vs. S
Dry matter						
a (%)	33.7	30.0	31.0	0.769	0.002	0.397
b (%)	44.3	48.2	45.6	0.727	<0.001	0.451
u (%)	22.0	21.8	23.4	0.688	0.817	0.079
k_d (% h^{-1})	6.51	6.54	4.84	0.309	0.947	<0.001
ED03 (%)	63.5	62.1	58.6	0.572	0.092	<0.001
ED06 (%)	56.3	54.3	50.9	0.656	0.033	<0.001
Nitrogen						
a (%)	45.4	40.9	59.7	1.730	0.075	<0.001
b (%)	38.3	42.4	21.9	1.367	0.038	<0.001
u (%)	16.4	16.7	18.5	0.561	0.666	0.007
k_d (% h^{-1})	13.3	11.1	11.0	0.644	0.020	0.155
ED03 (%)	76.5	73.9	76.5	0.557	0.002	0.064
ED06 (%)	71.7	68.0	73.5	0.663	<0.001	<0.001

a = soluble fraction; *b* = non-soluble degradable fraction; *u* = undegradable fraction; k_d = fractional degradation rate of fraction *b*; ED03 and ED06 = effective degradability using k_p of 0.03 and 0.06% per h, respectively; SEM = standard error of the mean; F = fresh forage; W = wilted forage; S = ensiled forage.
[a] Probability of the orthogonal contrast.

because that average was for material dried at 80 °C. The SP was quite variable between samples, (min: 17.68%; max: 35.0% for F), and was not correlated to CP content of F (r = 0.468; *P* = 0.290).

The in situ degradability of N from fresh forage (ED03 = 76.50% and ED06 = 71.65%) was similar to the 70–75% observed by Hoffman et al. [10] for fresh legumes harvested at mid-bloom stage.

The wilting effect on chemical composition was studied by Michalet Doreau and Ould-Bah [14]. These authors observed a significant increase of nearly 4% in NDF and ADF contents after 3 to 10 days of field drying of forage. In the present work, after 7.8 h of field drying, the magnitude of the increase was similar, but not significant. Alzueta et al. [1], studying chemical changes in field dried forage observed losses in non-structural carbohydrates as a result of plant respiration. These types of losses may have been responsible for the diminution in the DM soluble fraction (*a*) and the subsequent increase in the DM *b* fraction.

Wilting reduced effective degradability of N as a result of an increase in the non-soluble degradable fraction (*b*) with a decrease in fractional degradation rate (k_d). This fact was not accompanied by chemical changes, although NDIN-ADIN, tended to be higher in wilted than in fresh forage (*P* = 0.087, Tab. III). According to Licitra et al. [12] the nitrogen fraction insoluble in the neutral detergent solution, but soluble in acid detergent (NDIN-ADIN) is slowly degraded in the rumen. Alzueta et al. [1] found no significant changes in the chemical nitrogen fractions of vetch over two years, although an increase of non-protein nitrogen in field dried forages was observed. However, these authors did not study the degradation characteristics of the forages.

Likewise, Messman et al. [13], studying wilting periods of 24 hours in laboratory conditions reported little changes in the relative amounts of proteins identified after wilting forages.

The fermentation and storage processes produced a reduction in the effective degradability of DM. Aufrère et al. [3] comparing in situ degradation of direct-cut and ensiled lucerne, observed similar effects on the effective degradability associated with a higher undegradable fraction (*u*). These authors somehow attributed these changes to losses of volatile DM during freeze drying of silages, since the *a* and *b* fractions decreased, whereas the fractional degradation rate did not change. In the present work, a lower ED of DM of ensiled forage was mainly due to the lower fractional degradation rate of *b*, since neither fraction *a* nor fraction *b* changed. The lower k_d observed in ensiled material can be related to an increase in cell wall content. As shown in Table II, the composition of the silages was on average higher in NDF and ADF contents, the fractions that are slowly degraded in the rumen [16, 18].

Fermentation and storage transformed part of the nitrogenous components from the non-soluble degradable fraction (*b*) to the soluble (*a*) and undegradable (*u*) fractions. An increase in the *a* fraction is consistent with the increase in the SP chemical fraction [12], and may be evidence of protein degradation and deamination in the silos. On the contrary, the increase in the undegradable fraction was accompanied by an increase in ADIN, and indicates an increase of protein linked to cell walls due to Maillard reactions [21]. In spite of these results, no significant correlations could be detected within each treatment (F, W or S), -except a correlation between SP and *a* for wilted forage (r = 0.803; $P = 0.03$)-, perhaps because of the low number of samples per treatment (n = 7).

Aufrère et al. [3] observed similar changes in *a*, *b* and *u* of nitrogenous compounds during the ensiling process, even though effective degradability was not altered. These authors discussed that the degradability of fresh forages and silages does not differ because, as they observed in another study [4], the protein degraded during the fermentation process (rubisco: ribulose 1–5 diphosphate carboxylase), is the same as the protein degraded rapidly in the rumen in fresh forage. In the present work, when F and S were directly compared by analysis of variance, no significant differences were detected for ED06 (SEM = 1.246; $P = 0.087$), or ED03 (SEM = 1.100; $P = 0.998$). This comparison was made by Aufrère et al. [4], and the results were similar. However, the most important changes in ED occurred during wilting with a reduction in ED. This is the reason why ED of F+W is significantly lower when it is compared with S (Tab. IV).

The highest increase in the *a* fraction during fermentation and storage (silage/fresh ratio = 1.88) of the pastures included in the study, corresponded to No. 5 (Tab. I), which had the highest percentage of legumes (67.6% of *Trifolium pratense*). However, non-significant linear correlations were observed between botanical composition and conservation of the pastures.

5. CONCLUSION

It is concluded that wilting affected DM and N degradability of temperate forages, but did not change the chemical parameters studied. Meanwhile, fermentation and storage generated changes in N degradability with an increase in the soluble and non-degradable fractions. This was accompanied chemically by an increase in SP and ADIN. Further studies are needed to identify the causes of the changes that occurred.

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