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Wilting effect on fermentation characteristics and nutritive value of mountain permanent meadow grass silage

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Abstract – Grass from a pre-alpine permanent meadow located in the Cansiglio Highland (north Italy) was ensiled immediately after harvesting (T00), and after 5 h (T05) and 26 h (T26) of wilting. Two types of experimental silos were used to assess the chemical characteristics and the evolution of fermentation processes in pre-wilted silages: 500 L capacity silos (S500), opened after 6 months, and 2 L mini-silos (S002), opened at different times after ensiling (0, 12, 24, 48, 96 and 192 h). The forages ensiled in S500 silos were also fed to sheep in order to assess their digestibility and palatability. Dry matter (DM) concentration of fresh grass increased with wilting from 303 to 568 g/kg, while the concentration of other nutrients was scarcely affected by pre-wilting. Fermentation characteristics were significantly modified by wilting with an increase in pH (from 3.82 to 4.33) and a concurrent reduction in fermentation activity. All the fermentation end-products significantly decreased in the wilted silages: lactic acid decreased from 51.6 to 13.1 g/kg DM. Wilting did not affect the extent of fermentation losses. The fermentation processes in the short term were influenced by wilting with a slower development taking place in the wilted silages. The dry matter digestibility and the nutritive values of wilted silages were higher than those of unwilted silages (dDM: 0.52 in T00 to 0.58 in T26 silages), while voluntary intake of DM was not modified by wilting. (© Elsevier / Inra)

mountain meadow grass / wilting / ensiling / nutritive value

Résumé – Effet du préfanage sur les caractéristiques de la fermentation et la valeur alimentaire d'une prairie permanente de montagne. Trois tonnes d'herbe provenant d'une prairie sur le haut plateau du Cansiglio (Belluno, Italie du Nord) ont été ensilées immédiatement après la récolte (T00), et après 5 h (T05) ou 26 h de préfanage en plein air en juillet. Deux types de silos expérimentaux ont

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été utilisés pour évaluer respectivement les caractéristiques chimiques et l'évolution des processus de fermentation dans les ensilages préfanés : 12 silos ayant une capacité de 500 L (S500) (quatre silos par lot), ouverts après 6 mois, et 54 mini-silos ayant une capacité de 2 L, ouverts à différents moments après l'ensilage (0, 12, 24, 48, 96 et 192 h) (trois mini-silos par lot pour chaque temps). Les fourrages ensilés dans les silos S500 ont été donnés à 15 moutons (cinq par lot) afin d'en mesurer la digestibilité, la consommation volontaire et la valeur nutritive. La teneur en matière sèche (MS) de l'herbe fraîche a augmenté de 303 (T00) à 568 $g \cdot kg^{-1}$ (T26) avec le préfanage mais les autres éléments nutritifs ont été peu modifiés. Les caractéristiques de fermentation ont été influencées significativement par le préfanage : le pH a augmenté (de 3,82 à 4,33) et l'activité de fermentation a été réduite ; l'acide lactique a diminué de façon significative de 51,6 (T00) à 13,1 $g \cdot kg^{-1}$ MS (T26) ainsi que les concentrations en acide acétique et en azote ammoniacal. Le préfanage n'a pas affecté les pertes pendant les 6 mois d'ensilage, tandis que l'évolution des processus de fermentation durant 162 h après l'ensilage a été plus lente dans les ensilages préfanés. La digestibilité et la valeur alimentaire des ensilages préfanés ont été plus élevées que celles de l'ensilage non traité (dMS : de 0,52 dans l'ensilage T00 à 0,58 dans l'ensilage T26). En revanche, l'ingestion volontaire n'a pas été modifiée par le préfanage. En conclusion, le préfanage peut améliorer la qualité de conservation de l'ensilage, ainsi que sa valeur alimentaire. Cette dernière observation paraît être en contradiction avec plusieurs études qui ont montré une réduction de la qualité nutritionnelle des ensilages préfanés. (© Elsevier / Inra)

prairie permanente de montagne / préfanage / ensilage / valeur nutritive

1. INTRODUCTION

Forage production in hill and mountain areas is limited by the low quality of grass produced, mostly as hay. Many factors influence hay quality, in particular meteorological conditions during grass harvesting and field wilting, and the slow growth of the grass in spring. These conditions result in the grass being of low nutritional quality and are responsible for high losses in both the quantity and quality of hay.

The pre-alpine area (800–1500 m above sea level) of north-eastern Italy is characterized by high annual rainfall (1500–2000 mm), with abundant rain in April and May and frequent showers in summer. This situation permits only one harvest of grass to be field cured in the period from mid-July to mid-August, and sun drying often requires 4 days or more because of frequent showers, especially in the afternoon.

The ensiling of grass can represent a valid alternative to hay-making due to the better nutritional and galactopoietic properties of grass silages. The principal drawback to the ensiling of grass is its moisture content, which is nearly always too high to guarantee the correct development of fermentation processes. The wilting of fresh herbage prior to ensiling can be helpful in this respect.

During the ensiling of pre-wilted forages, the lower moisture content reduces microbial activity, protein degradation, and fermentation losses, and prevents effluent losses [12, 15, 21].

The experimental results on the effects of pre-wilting on the nutritive value and livestock performance of silage are rather contradictory. Gordon [6] and Thomas and Thomas [21] reported that pre-wilted silages showed lower apparent digestibilities than unwilted silages, even though the voluntary intake was generally stimulated following wilting. On the contrary, Yan et al. [28] observed higher digestibility when animals were fed pre-wilted silages. As reviewed by Gordon [6], animal performances usually appear to be affected negatively by pre-wilting, whereas other authors describe positive or unaffected results [5, 8, 19].

The aim of the present research was therefore to evaluate the effect of different pre-wilting periods on the fermentation processes, preservation characteristics, and nutritive value of silages obtained from permanent meadow grass in pre-alpine areas.

2. MATERIALS AND METHODS

2.1. Herbage harvesting and silo preparation

The permanent meadow, located in a farm owned by E.S.A.V. (Agriculture Development Agency of Veneto Region) in the pre-alpine area (Vallorch, Cansiglio Highland, 1000 m above sea level) was cut at full blooming. The meadow was characterized by a high content of grass species (about 60%, in particular *Phleum pratense* and *Festuca rubra*), a limited presence of legume species (about 10%, mainly *Trifolium pratense*) and a wide range of other families (Cruciferae, Compositae, Polygonaceae, etc.).

Three tons of herbage were harvested during the second week of July. The grass was cut between 09.30 to 10.00 a.m. using a horizontal rotary mower from an area of about 2000 m^2. The grass was lifted from the field in alternate rows at three times using a green crop cart with a front pick-up loader: one third of the herbage was loaded immediately (treatment T00); one third was left in the field for 5 h without tedding (treatment T05); one third was left to wilt until 5.30 p.m., then windrowed for the night; the windrow was spread the next day at 09.30 a.m. and the grass was picked up at 11.30 a.m. after an overall pre-wilting period of 26 h (T26). Before cutting and during wilting, weather conditions were stable and sunny, with high temperature and low humidity.

Immediately after harvesting, the herbage was moved to a roofed shelter where it was chopped using an electric straw cutter. The chopped material was then ensiled in 12 experimental silos (four silos for each pre-wilting treatment). These silos had 500 L capacity (S500), a hermetic cover, and a valve at the bottom to permit the recovery of effluents. These silos were stored in a shed and opened 6 months later.

At the same time, 54 mini-silos of 2 L capacity, consisting of glass jars for food preserves equipped with hermetic covers, were filled (S002). These silos, stored in the same shed of the S500, were opened at different times after ensiling: 0, 12, 24, 48, 96 and 192 h (three mini-silos × three wilting treatments × three ensiling times).

More detailed information on ensiling technique and experimental silo typology has been provided in previous papers [25, 26].

2.2. Digestibility trial and voluntary intake

Silages stored in the S500 silos were fed consecutively to 15 ewes of Finnic or Lamon breed (five animals per pre-wilting treatment) during a digestibility and voluntary intake trial carried out following the methodology suggested by the Associazione Scientifica di Produzione Animale [1].

Each group of ewes was fed with silage (40 g DM/day/kg $LW^{0.75}$ of silage) and trace mineral-vitamin supplement (50 g/day) during a 21-day preliminary period. The same diet was fed throughout the 7-day collection period, during which faeces were collected daily for each individual animal (10% of total weight) and stored at –18 °C. At the end of the digestibility trial, voluntary intake was measured for 7 days following an adaptation period of 4 days in which the animals were fed ad libitum. This adaptation period was sufficient to reach a constant voluntary intake in the following 7 days.

At the end of the digestibility trial, two samples (200 mL) of rumen liquor were taken from each ewe by an esophagus probe connected with a vacuum pump: the first sampling immediately after feeding and the second 3 h later.

2.3. Sample collection and analytical procedures

The fresh material was sampled when the experimental silos were prepared, taking nine samples for each S500 silo. When the experimental silos were opened, nine samples per S500 silo and 1 sample per S002 silo were immediately taken. After DM determination, the samples taken from the S500 silos were bulked to provide three samples for further analyses (three samples per silo).

Fresh grass, silages, faeces and rumen liquor were analyzed following the methods previously described [26].

The DM content and the chemical composition of the silages were corrected according to their concentrations of organic acids, alcohols and ammonia-N as suggested by Dulphy and Demarquilly [4].

The apparent digestibility coefficients of the various nutrients and energy in the experimental diets were calculated on the basis of the ingesta-excreta balance and the chemical composition of foods and faeces. The nutritive value of the silages was estimated from the proximate chemical composition and the in vivo apparent digestibility coefficients as described by Vermorel [24].

The experimental results were submitted to analyses of variance using the least squares mean method and the LSMLMW program [9]. For the statistical analyses of the fermentation characteristics, ensiling losses, and in vivo digestibility coefficients, a linear model that considered wilting period (W) as a principal effect was utilized. In addition to this effect, the analyses of the progress of fermentation characteristics in the S002 silos also included the 'time after ensiling' effect (T) and the interaction (W × T) effect.

3. RESULTS

3.1. Chemical composition of fresh and ensiled forages

The stage of growth of herbage at cutting was quite advanced, as demonstrated by the high DM concentration (303 g/kg) and the fairly high level of NDF (669 g/kg DM) of the fresh material (treatment T00) (*table I*). The degree of lignification of grass was still moderate, but the protein concentration was low (84 g/kg DM), as a consequence of the late harvesting of the meadow and the type of grass species present.

Owing to the good weather conditions during wilting, DM rose to 472 and 568 g/kg in T05 and T26 grasses, respectively. A reduction in the protein concentration was observed as the pre-wilting period increased (84 to 76 g/kg DM). The NDF concentration increased in the T26 forage, but this change was not confirmed by similar variations in other cell wall constituents.

On the whole, the chemical composition of the silages is consistent with that of fresh herbage at ensiling (*table I*). The ether extract concentration proved to be higher in all the silages when compared to fresh products, due to the accumulation of fermentation metabolites included in the ether-extract fraction [22] and decreased with the pre-wilting period.

3.2. Fermentation characteristics

Fermentation characteristics of silages stored for 6 months in S500 silos were generally good (*table II*).

As the herbage DM increased with wilting period, the final pH reached higher levels (3.82 vs. 4.04 vs. 4.33, in the T00, T05 and

Table I. Chemical composition of fresh grass and silages (means ± S.D.).

	Fresh grass			Silages[1]		
	T00	T05	T26	T00	T05	T26
Wilting (h)	0	5	26	0	5	26
No. of samples	12	12	12	12	12	12
DM(g/kg)	303 ± 14	472 ± 26	568 ± 33	297 ± 11	459 ± 18	569 ± 19
Crude protein (g/kg DM)	84 ± 4	79 ± 9	76 ± 6	83 ± 5	78 ± 7	76 ± 3
Ether extract (g/kg DM)	24 ± 4	20 ± 2	21 ± 2	38 ± 6	29 ± 5	25 ± 4
Ash (g/kg DM)	49 ± 5	48 ± 5	44 ± 2	50 ± 4	48 ± 3	45 ± 1
NDF (g/kg DM)	669 ± 13	677 ± 16	685 ± 20	622 ± 20	639 ± 11	647 ± 10
ADF (g/kg DM)	395 ± 9	396 ± 8	386 ± 8	387 ± 17	389 ± 15	394 ± 12
ADL (g/kg DM)	64 ± 4	65 ± 5	57 ± 3	56 ± 7	52 ± 10	55 ± 8

[1] Silages stored in 500 L capacity silos (S500).

Table II. Effect of length of wilting period on fermentation characteristics of the silages in the 500 L capacity silos (S500).

	Silages				Significance[1]	
	T00	T05	T26	s.e.d.	*L*	*Q*
Wilting (h)	0	5	26			
No. of silos	4	4	4			
pH	3.82	4.04	4.33	0.04	***	
Ammonia N (g/kg total N)	61.2	48.2	37.2	3.4	***	
Lactic acid (g/kg DM)	51.6	26.5	13.1	2.1	***	**
Acetic acid (g/kg DM)	21.6	16.3	7.9	1.5	***	
Propionic acid (g/kg DM)	1.1	0.4	0.5	0.5		
Butyric acid (g/kg DM)	0.9	0.1	0.1	0.4	*	
Ethanol (g/kg DM)	9.4	5.3	3.6	0.4	***	***
Mannitol (g/kg DM)	30.6	28.4	23.0	2.1	***	
Glucose + fructose (g/kg DM)	23.6	30.4	39.1	3.7	***	
Homolactic index[2]	1.73	1.28	1.06	0.10	***	
Flieg score[3]	86	90	90			

[1] *L*, linear component of variance; *Q*, quadratic component.
[2] Lactic acid/(acetic acid + ethanol) ratio.
[3] Flieg score as modified by Vanbelle and Bertin [23].

T26 silages, respectively), while organic acid production, especially that of lactic acid, decreased ($P < 0.01$). The activity of proteolytic micro-flora and the consequent ammonia-N concentration decreased (from 61.2 to 37.2 g/kg of total N, $P < 0.001$), along with both butyric and propionic fermentation. The production of ethanol was similarly reduced by wilting, while the production of the most prevalent alcohol, i.e., mannitol, was less affected.

In T00 silage, homolactic fermentation appeared to be predominant in comparison with the heterolactic fermentation: the homolactic index, i.e., the ratio between lactic acid and the sum of acetic acid and ethanol [26], was 1.73, whereas it decreased to 1.28 and 1.06 in T05 and T26 silages, respectively.

The Flieg score, as modified by Vanbelle and Bertin [23], was always 'very good' regardless of the wilting treatment.

3.3. Ensiling losses

No effluent losses were observed during the storage of any of the silages.

The higher fermentation losses observed in the T00 and T05 silages than in T26 silage (*table III*) suggested a trend, but statistical analysis did not reveal any significant differences among treatments. Only ether extract gain was significantly reduced in wilted silages because of the decrease in fermentation activity and as outlined earlier the inclusion of organic acids in the ether extract fraction. On average, hemicellulose was more degraded than other chemical constituents (0.14 to 0.17).

Wilting modified the density of herbage within the silos: from 0.45 g/cm^3 in T00 silage to 0.28 g/cm^3 in T26 silage. This was brought about by the length of chopping and limiting the compression at ensiling.

Table III. Effect of length of wilting period on density (fresh basis) and ensiling losses of the silages in the 500 L capacity silos (S500).

	Silages				Significance[1]	
	T00	T05	T26	s.e.d.	*L*	*Q*
Wilting (h)	0	5	26			
No. of silos	4	4	4			
Density (g/cm^3)	0.45	0.33	0.28	0.01	***	***
Ensiling losses						
Dry matter	0.030	0.037	0.012	0.029		
Organic matter	0.031	0.037	0.012	0.030		
Crude protein	0.045	0.049	0.011	0.023		
Ether extract	–0.558	–0.354	–0.172	0.138	*	
NDF	0.099	0.092	0.066	0.030		
Hemicellulose	0.169	0.144	0.163	0.041		

[1] *L*, Linear component of variance; *Q*, quadratic component.

3.4. Evolution of fermentation process over time

The fermentation characteristics of the silages stored in the experimental mini-silos (S002) during 8 days after ensiling are listed in *table IV*.

Given that no significant interaction between the effect of the wilting period (W) and hours after ensiling (T) was observed, the general evolution of the fermentation processes can be described by the mean values of the principal effects.

Wilting period significantly affected ($P < 0.001$) all fermentation characteristics (mean values of the six opening times), except for the production of butyric acid (< 0.01 g/kg) and propionic acids. The fermentation patterns observed only a few days after ensiling clearly reflected the final characteristics measured in the mature silages 6 months later (S500 silos). The increase in DM concentration due to wilting resulted in a higher pH and a lower concentration of fermentation end-products in comparison with unwilted silage, while glucose and fructose were less fermented.

The effect of hours after ensiling was even greater than that of wilting, and led to a sharp variation in pH and end-product concentrations (mean values of three wilting treatments). Fermentation activity was weak in the first 12 to 24 h, and particularly intense during the following 24 h: in the second day, pH decreased quickly (4.64 on average), while organic acids rapidly increased (*figure 1a, b, c*). Both lactic acid and acetic acid reached their maximum rate of increase after around 48 h, with a clear slowdown after 96 h.

The production of ethanol and mannitol followed different trends: a rather high concentration of ethanol was achieved within 48 h after ensiling, while mannitol production was appreciable only after 48 h (*figure 1d, e*).

Proteolysis was apparent from the second day of ensiling (*table IV, figure 1f*), as were both lactic and acetic production.

Table IV. Effect of length of wilting period (W) and time after ensiling (T) on the fermentation characteristics of the silages in the 2 L capacity silos (S002)

	Wilting (W)				Time after ensiling (h) (T)							Significance[1]		
	T00	T05	T26	s.e.d.	0	12	24	48	96	192	s.e.d.	W_L	T_L	T_Q
No. of silos	18	18	18		9	9	9	9	9	9				
DM (g/kg)	303	400	567	7	449	462	453	456	446	454	10	***		
pH	4.79	5.00	5.21	0.05	5.68	5.63	5.09	4.64	4.51	4.44	0.07	***	***	***
Ammonia N (g/kg total N)	20.9	12.7	8.0	0.8	1.7	5.9	9.9	16.3	21.6	27.9	1.1	***	***	***
Lactic acid (g/kg DM)	14.1	5.5	2.7	1.1	0.1	0.4	2.0	8.1	14.6	19.4	1.5	***	***	***
Acetic acid (g/kg DM)	5.4	2.9	1.9	0.3	0.4	0.5	1.5	3.7	6.4	7.8	0.3	***	***	***
Propionic acid (g/kg DM)	0.1	0.1	0.1	0.03	0.0	0.1	0.2	0.1	0.1	0.1	0.04			
Ethanol (g/kg DM)	5.2	2.7	2.3	0.2	0.5	2.0	3.1	4.1	5.2	5.4	0.3	***	***	***
Mannitol (g/kg DM)	12.5	9.2	3.7	0.6	0.0	0.0	0.0	8.4	16.3	26.0	0.9	***	***	***
Glucose + fructose (g/kg DM)	37.9	43.6	55.0	2.7	53.5	55.3	55.2	41.8	35.3	31.6	3.9	***	***	***
Homolactic index	0.45	0.34	0.33	0.06	0.01	0.15	0.37	0.57	0.54	0.53	0.09	*	***	***

[1] W_L, linear component of W variance; T_L, linear component of T variance; T_Q, quadratic component of T variance.

3.5. Digestibility, nutritive value and rumen fluid composition

Digestibility coefficients and nutritive value of the three silages are shown in *table V*.

The unwilted silage had low apparent digestibilities of DM, energy and crude protein, whereas the digestibility of cellulose was higher. As a consequence, the estimated nutritive value was moderate, slightly lower

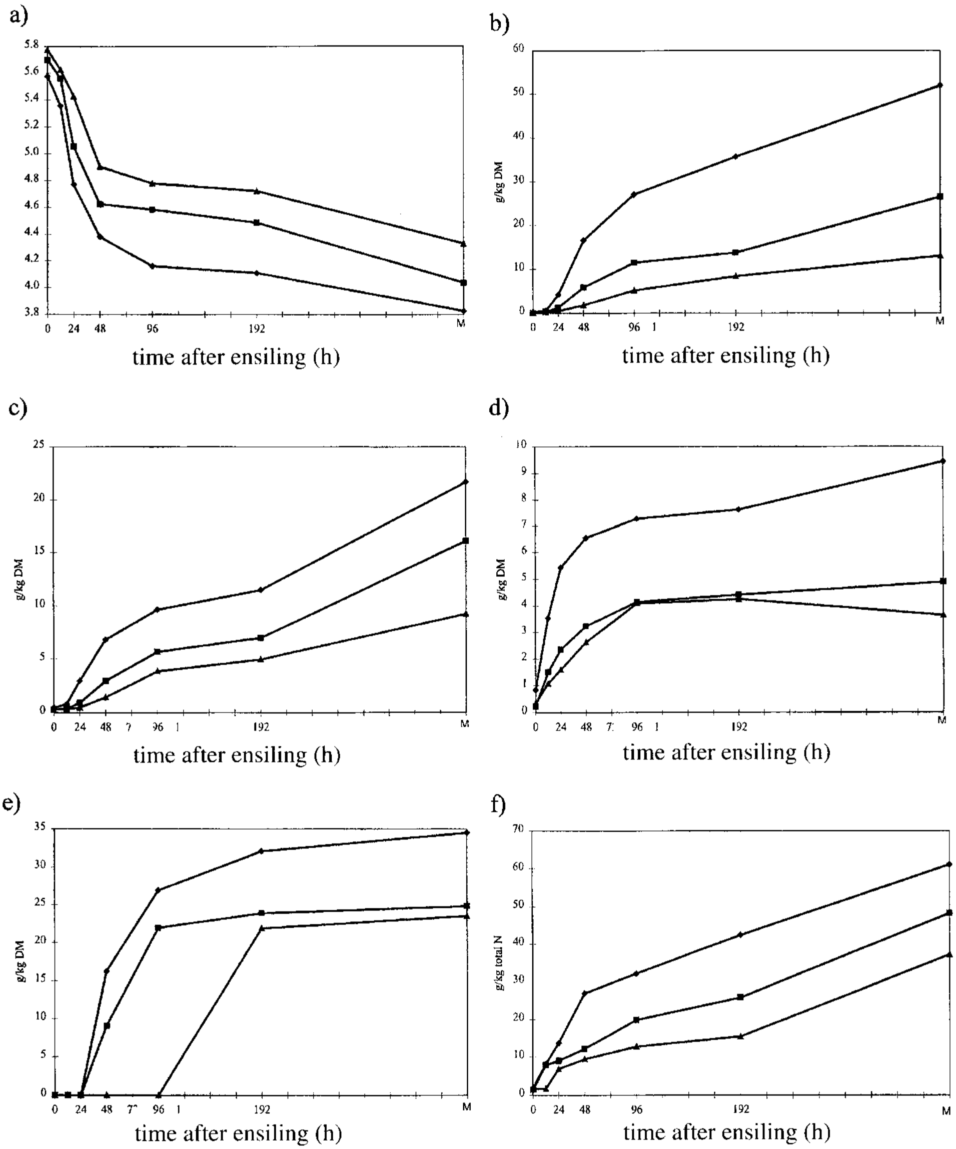

Figure 1. Changes in pH (**a**), lactic acid (**b**), acetic acid (**c**), ethanol (**d**), mannitol (**e**) and ammonia-N (**f**) until 192 h after ensiling in the S002 silos compared to mature silages (M) in the S500 silos (◆ T00; ■ T05; ▲ T26).

Table V. In vivo digestibility, voluntary feed intake and estimated nutritive value of the silages.

	Silages			Significance[1]		
	T00	T05	T26	s.e.d.	*L*	*Q*
Wilting (h)	0	5	26			
No. of ewes	5	5	5			
Digestibility coefficients						
DM	0.52	0.55	0.58	0.02	**	
Organic matter	0.53	0.56	0.58	0.02	**	
Crude protein	0.33	0.42	0.44	0.04	*	
NDF	0.46	0.51	0.54	0.02	**	
Hemicellulose	0.46	0.52	0.54	0.02	**	
ADF	0.45	0.51	0.54	0.03	**	
Cellulose	0.61	0.61	0.64	0.02		
Energy	0.49	0.53	0.54	0.02	*	
DM voluntary intake						
g/d	1 060	1 006	1 090	135		
g/kg $LW^{0.75}$	47.2	44.5	48.2	4.8		
Nutritive value						
DE (MJ/kg DM)	8.81	9.36	9.57			
ME (MJ/kg DM)	7.18	7.60	7.74			
UFV (no./kg DM)	0.46	0.50	0.52			
UFL (no./kg DM)	0.56	0.60	0.61			

[1] *L*, linear component of variance; *Q*, quadratic component.

than medium-quality hay. The voluntary intake of DM (47.2 g/day/kg $LW^{0.75}$) was slightly higher than the restricted intake of the digestibility trial (40 g/day/kg $LW^{0.75}$).

Wilting significantly improved the apparent digestibility of DM (from 0.52 in T00 to 0.55 and 0.58 in T05 and T26 respectively, $P < 0.01$) and almost all nutrients. The estimated nutritive value was consequently higher in the wilted silages (0.61 UFL and 0.52 UFV/kg DM in T26 vs. 0.56 UFL and 0.46 UFV in T00). The voluntary intake of wilted silages, on the other hand, was not significantly influenced by wilting.

Rumen fluid composition varied between the silages (*table VI*). As the length of wilting period increased, rumen fluid pH decreased significantly while total VFA concentration rose ($P < 0.10$). The rumen liquor of ewes fed wilted silages contained a slightly lower concentration of propionate ($P < 0.05$) and valerate ($P < 0.01$), while butyrate concentration was stimulated by wilting. The acetate: propionate ratio consequently increased slightly with wilting (from 3.13 to 3.21, and 3.48; $P < 0.10$).

4. DISCUSSION

4.1. Effect of wilting on chemical composition of grass

Apart from DM concentration, in the present study there were very few changes in chemical composition caused by pre-wilting prior to ensiling. In fact, DM concentration of grass increased quickly and a slight decrease in its protein concentration was observed even after 26 h. This decrease is

Table VI. Molar proportions of volatile fatty acids in the rumen fluid of sheep 0 and 3 h after being fed the silages.

	Wilting (W)				Sampling time (S)			Significance	
	T00	T05	T26	s.e.d.	0 h	3 h	s.e.d.	W[1]	S
No. of samples	10	10	10		15	15			
pH	6.99	6.85	6.68	0.10	7.02	6.66	0.08	**	**
Total VFA (mmol/L)	57.9	65.8	63.3	4.3	53.1	71.6	3.5		**
Acetate (% total VFA)	66.7	67.0	67.3	1.5	71.1	62.9	1.2		**
Propionate (% total VFA)	22.0	21.3	19.9	1.0	19.0	23.1	0.8	*	**
Butyrate (% total VFA)	8.4	9.5	10.6	0.8	7.9	11.1	0.6	**	**
Valerate (% total VFA)	2.9	2.3	2.2	0.2	2.1	3.0	0.1	**	**
Acetate:propionate ratio	3.13	3.21	3.48	0.19	3.78	2.77	0.16	< 0.10	**

[1] Linear component of variance.

likely to be due to mechanical losses (legume leaves). The rate herbage DM changes during wilting basically depend on weather conditions, while changes in chemical composition do not appear to be directly influenced by pre-wilting treatment [10, 14]. Nevertheless, some authors observed that a long pre-wilting period can have a negative effect on the composition and ensiling suitability of herbage, especially when the weather is poor. This latter effect is due to the respiration and proteolytic processes that cause an appreciable reduction in water-soluble carbohydrates and an increase in ammonia-N concentration [10, 12]. Kowalski et al. [11] and Yan et al. [28] found an appreciable increase in the protein concentration of wilted silages. In view of these possible effects, it is obviously preferable that wilting time prior to ensiling be as short as possible [19, 28].

4.2. Effect of wilting on fermentation processes and ensiling losses

Fermentative activity was considerably reduced by wilting, with changes during ensiling directly dependent on the variations of the initial herbage DM level.

Previous reviews of studies concerning the effect of wilting on silage quality showed a decrease in ammonia-N concentration in wilted silages [11]. Using unwilted (DM 206 g/kg), 24-h wilted (DM 346 g/kg) and 48-h wilted silages (DM 513 g/kg), inoculated or not with *Lactobacillus plantarum*, O'Kiely [17] observed an increase of ammonia-N production after 24 h of wilting (from 88 to 103 g/kg total N), followed by a decrease after 48 h (78 g/kg total N). Also, Charmley et al. [3] and Yan et al. [28] observed an increase in ammonia-N in some high-moisture harvests following wilting.

As regards the effect of initial moisture on the development of different bacteria involved in fermentation processes, our results demonstrated the virtual absence of clostridia activity in all silages (butyric acid < 1 g/kg DM) and the reduction in lactic acid concentration was proportional to the increase of DM in the wilted silages but still sufficient to guarantee a suitable reduction in pH. In fact, lactic acid bacteria appear to be less sensitive to increases in DM concentration than clostridia [12], which are virtually inhibited when the herbage DM is over 300 g/kg.

Some authors suggested that wilting can also modify the ratio between homofermentative and heterofermentative lactic species, but opinions on this subject vary widely. Muller et al. [16] observed the prevalence of homolactic bacteria in respect to heterolactic strains, probably due to the different degree of osmotolerance. On the other hand, McDonald et al. [12] suggest that 98% of the lactic bacteria species in wilted silage are heterofermentative. The change in the homolactic index observed in our experiment seems to support the latter hypothesis.

On the whole, the high initial DM in all fresh herbages severely limited effluent and fermentation losses as these are appreciable only with initial DM less than 250 g/kg [2, 26].

Wilting reduced silage density, and this might increase respiration losses [13, 15]. According to our results however, the decrease in fermentative activity amply compensates for the less favorable anaerobic conditions.

Results from the S002 mini-silos showed that fermentation processes differed significantly even only a few hours after ensiling. All the fermentation processes developed along similar pathways in the three silages but at lower intensities as wilting duration increased. Fermentation activity peaked between 24 and 96 h and then diminished. All these fermentation pathways lasted a minimum of 192 h, even with progressively diminishing intensities and did not stop completely, as evidenced by the higher end-product levels reached in the mature silages. The differences between certain fermentation characteristics in the S002 silos opened after 192 h, and the S500 silos opened after 6 months, could be ascribed not only to the slow fermentation process, but also to the different size and sealing system of the experimental silos [25, 26]. Mannitol production was appreciable only after 2 days of ensiling and followed patterns already observed by other authors [13, 27].

4.3. Effect of wilting on digestibility, nutritive value and rumen fluid composition

Unlike our results, many of the studies available [3, 6, 19, 21] demonstrated negative effects of wilting on the digestibility of silages by ruminants and ascribed them to the increase of nutrient losses in the field due to respiration.

In our trial no negative effect of wilting on field losses of nutrients was recorded, while the more intense fermentation activity of the unwilted silage could account for its lower nutritive value. In fact, if we calculate the concentration of indigestible protein in T00, T05 and T26, we find 55.6, 45.2 and 42.6 g/kg DM, respectively. The value observed in T00 silage is unusually high, probably as a consequence of silage heating in the first phase of ensiling. This hypothesis is consistent with the evolution of fermentation processes we observed in the short term period.

Gordon and Peoples [7] reported lower nutrient digestibility in lactating cows fed silages cut from perennial ryegrass swards wilted under difficult weather conditions than unwilted silages, but an opposite tendency was observed in a similar experiment when wilting was achieved under sunny conditions [20]. Yan et al. [28] observed both positive and negative effects of wilting on the digestibility of perennial ryegrass silage depending on the harvest period. Both weather conditions and initial herbage DM, as in our trial, may modify the final DM after wilting with a positive effect on the conservation processes and therefore the nutritive value of silage.

As regards the voluntary intake of DM, the absence of difference between unwilted and wilted silages observed in our study contrast with observations made by other authors [5, 8] who reported a higher DM intake with wilted silages. However, in spite of the higher intake with wilted silage, this is often accompanied by a worsening of the overall animal performance [6, 21]. According to Patterson et al. [19], the decrease in the performance of animals fed wilted forages may depend on wilting management, since the longer the forage stays in the field, the higher the nutrient losses.

In our study, the variation in rumen fluid composition may partially explain the higher nutritive value calculated for wilted silages, which stimulated higher butyrate concentrations than unwilted silages. Similar observations were made by Peoples and Gordon [20] using silages wilted under favorable conditions, while Gordon and Peoples [7] did not find different rumen VFA concentrations in heifers fed silages from perennial ryegrass wilted under difficult conditions. In the rumen fluid of steers fed unwilted silages enriched with formic acid, O'Kiely and Flynn [18] found a lower acetate: propionate ratio than in steers fed wilted silages.

This study confirmed wilting as a pre-ensiling treatment which is capable of improving the chemical characteristic of silages by reducing fermentation activity, proteolysis and storage losses even when the grass is harvested at a late stage of maturity (DM 300 g/kg). Ensiling after wilting appears to be a useful practice that should be applied in mountain areas, where the nutritive value of the permanent meadow grass is normally low and negatively affected by unfavorable weather conditions during haymaking.

Under these conditions, a short pre-wilting period improved the nutritional properties of the silages, since digestibility and hence nutritive value were observed to be significantly higher in wilted than unwilted silages. Better results were achieved when the wilting was extended for 26 h instead of 5 h.

In contrast with some previous studies that documented a reduction in nutritive value in wilted silages, our results probably derive from the sunny weather during wilting and the high initial DM of herbage. These conditions contributed to a very rapid achievement of the moisture levels suitable for the correct evolution and orientation of the fermentation processes, by reducing the heating of the silage, and the improvement of nutrient utilization in some way. However, these observations should be further investigated by testing the effect of wilting on permanent meadow grass harvested at different stages of maturity.

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