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## Ensiling Alfalfa (*Medicago sativa* L.) and Orchard Grass (*Dactylis glomerata* L.) Forage Harvested at 08:00 or 14:00, without Wilting or 1 or 2 h Wilting and with or without Use of Bacterial Inoculant

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Additional information is available at the end of the chapter

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### Abstract

Alfalfa forage is difficult to ensile due to low water-soluble carbohydrate content and high buffering capacity. The objective was to assess at Chapingo, Mexico, during the rainy season effects of combinations of harvest hours (08:00, 14:00), wilting time (0, 1, 2 h) and bacterial inoculants on the quality of silage made of alfalfa and orchard grass forage, made in 200-L containers. The experiment was conducted in three phases with two replicates per phase. Variables measured in freshly cut forage and silages were dry matter content (DM), buffer capacity, pH, and alcohol soluble carbohydrates (ASC). Silos remained sealed during 60 d, and additional variables measured in silage were aerobic stability, NH<sub>3</sub>-N and in vitro disappearance of DM. In forage harvested at 14:00 h, DM and ASC contents were higher; pH and buffering capacity were not affected by harvest hour; in silages made of that forage, NH<sub>3</sub>-N levels were lower, while ASC contents and in vitro disappearance of MS were unaffected by harvest hour. Treatments with inoculants were less aerobic stable for 5 days when made of forage harvested at 08:00 h but more stable when made of forage harvested at 14:00 h. Harvesting at 14:00 h was advantageous as silage presented higher DM and ASC contents.

**Keywords:** silage, *Medicago sativa*, wilting, inoculant, harvesting time

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## 1. Introduction

Alfalfa (*Medicago sativa* L.) grasslands are dominant in irrigated temperate Mexico; however, their yield in the dry autumn-winter season is 50% lower than during the rainy spring-summer [1]. Conservation of forage surpluses is therefore necessary; but, due to summer rains, haymaking is risky and hence silage making becomes the best option. Nonetheless, ensiling alfalfa forage is difficult because of its low content of water-soluble carbohydrates and high buffering capacity, which delay the lactic acid production and therefore hinder a rapid decline of pH within the ensiled forage [2].

Technical alternatives for ensiling alfalfa forage are to harvest in afternoon hours [3], wilting [4] and the use of lactic acid bacteria (LAB) homo- and heterofermentative inoculants with enzymes [5].

Cutting in the afternoon and wilting increased dry matter (DM) and alcohol soluble carbohydrates (ASC) concentration in alfalfa, which led to silage with improved conservation attributes such as lower pH, greater concentrations of lactate, lower concentrations of volatile fatty acids and NH<sub>3</sub>-N [3]. However, the effect of wilting largely depends on weather conditions, since events of rain during wilting, together with poor drying conditions (low potential evapotranspiration) may lead to considerable rise in pH and losses of sugars, causing worse silage fermentation [4].

The use of inoculants consisting of homofermentative bacteria hastened the drop of pH during the fermentation but did not improve the aerobic stability of the silage [6]. Such result is consistent with [7], who reported that inoculants based on homofermentative lactic acid bacteria did not improve aerobic stability in about two thirds of the cases; on the contrary, inoculant based on the heterolactic acid bacterium *Lactobacillus buchneri* increases the aerobic stability of silages mainly due to a rise in the concentration of acetic acid.

Aerobic stability is defined as the length of time that silage remains cool and does not spoil after it is exposed to air [7]. Since silage making relies on keeping anaerobic conditions, once the silo is open in the feeding phase and hence the silage is exposed to oxygen, it becomes liable to oxidation by the coming into action of dormant aerobic bacteria, yeasts and molds, which will be producing CO<sub>2</sub> [8], leading to rises in temperature and pH [7].

The fermentation and storage phases are dominated by anaerobic processes; as a result, silage contains yeasts, molds and some aerobic bacteria which are dormant under these anaerobic conditions. Introduction of oxygen, by deterioration of the sealing or opening the silo for the feeding phase, activates these aerobic microorganisms whose respiration consumes valuable nutrients, producing carbon dioxide and water with loss of dry matter and nutrients and decay of the silage [7].

Based on the above stated, this study was aimed at assessing during the rainy season, in three different months (different short-term weather conditions) the effect of hour at harvest, wilting and use of a mixed inoculant on quality properties of freshly cut forage from a mixed alfalfa and orchard grass (*Dactylis glomerata* L.) grassland dominated by alfalfa, and the corresponding silages.

## 2. Material and methods

The field work was carried out in three silage phases between June and September 2011 at Chapingo University, State of Mexico, Mexico, 19° 29 'N, 98° 54 'W and 2240 meters above sea level, under temperate sub-humid climate with summer rains.

Forage from a 2-year old alfalfa-orchard grass mixed grassland (0.37ha) was used, harvested after 42 days of regrowth, yielding on average 2480 kg DM/ha per harvest. Such mixed grasslands are regularly used within an irrigated grassland and forage crops rotation for dairy production under grazing; during the first 2 years of these grasslands, alfalfa is dominant (more than 70% of dry matter of harvested herbage). As silos, plastic containers with a capacity of 200 L, 52 cm in diameter and 95 cm in height were used, with lid and strap for air tight sealing.

The experiment comprised three phases (phase 1: June–July, phase 2: August, phase 3: September) in which 12 treatments were evaluated with two replicates in each phase. The treatments resulted from the combination of  $2 \times 2 \times 3$  complete factorial arrangement of two cutting schedules (08:00 and 14:00 h), two levels of LAB inoculant (0 and 5 g/t forage) and three wilting times (0, 1 and 2 h). During each day of operation, two containers were filled (one with forage cut at 08:00 h and the other with forage cut at 14:00 h), following a random order.

Forage was cut with a scythe, according to treatments it was wilted on the field, thereafter gathered and carried 300 m to the ensiling facility where it was cut into 3 cm (on average) particles using a Mapusa® (Pudong, Shanghai, China) mincer. During mincing the forage was covered with a polyethylene film to avoid dehydration and contamination.

The inoculant used was BIOTAL PLUS II® (Lallemand Animal Nutrition, Milwaukee, Wisconsin) that contained viable cells of the LAB *Pediococcus pentosaceus* 12,455 (homofermentative) and *Propionibacterium freudenreichii* R2453 (heterofermentative) and specific enzymes, which were expected to enhance fiber hydrolysis during ensiling [5]. The inoculant was applied following instructions; hence, it was expected to supply 100,000 CFU/gram of forage.

For the compaction of the forage a structure was designed consisting of two vertical concrete cylinders of 0.25 m in diameter and 2.8 m in height, separated 1.15 m and joined at the top by a steel crossbar of 7.62 cm diameter and 1.25 m long. A hook with a pulley was placed on the crossbar, which was used to compact forage, operating with a rope to vertically move a cement piston with a diameter of 49 cm, height 25 cm and weight of 53 kg. The controlled displacement of the piston inside the containers was stabilized by placing them inside a metal ring 40 cm high that had hinges to open it when entering or removing each container.

The forage to be ensiled was placed within the containers in layers of 5 kg extended with pitchfork and the inoculant was applied with atomizer; two operators used the rope and the pulley to raise and drop the piston 60 times on each forage layer; when the section to be filled with the container was of less height than the piston, a metal sheet tube 50 cm high and 50 cm in diameter was used as a guide for the falling piston. Once each container was filled, the lid was placed and sealed with adhesive tape and secured with the strap. The containers were weighed on a Trutest® (Auckland, New Zealand) scale to verify that density was within target ranges (resulting in  $608 \pm 31 \text{ kg m}^{-3}$ ) and thereafter placed outdoors for 60 days, after which they were opened for sampling.

## 2.1. Measured variables

With a Davis Instruments Vantage Pro2® (Hayward, California) meteorological station, temperature, rainfall and humidity data were recorded at the time of forage harvesting, wilting and ensiling; these data were used to unravel the effect of weather variables on silage properties. There were no clear differences among phases with on average 0.14 mm rainfall in the morning hours and much less in the afternoon hours (0.04 mm); on the contrary, evapotranspiration was low in the morning hours (on average 0.21mm) and higher in the afternoon hours (on average 2.54 mm). The containers were opened 60 days after ensiling, a silage top layer of 30 cm was removed, the temperature was measured, and samples were taken for determinations of pH, DM content, aerobic deterioration and chemical composition.

### 2.1.1. Morphological characterization and quality indicators of the forage before ensiling

In the forage to be ensiled samples were taken to measure botanical composition, and DM content, temperature, pH, ASC (alcohol soluble carbohydrates), and buffering capacity.

Botanical and morphological composition of forage was estimated by means of hand separation. The DM content was estimated by drying at 65°C to constant weight, this variable was estimated in freshly cut forage or after wilting according to treatments, and additionally at the beginning, half and end of the process of filling the containers.

Temperature and pH measurements were made with a portable Orion 3-Star® meter (Thermo Fisher Scientific Inc., Chelmsford, Massachusetts). The temperature was measured at the beginning, middle and end of the container filling process. For the pH measurement the samples of approximately 50 g of fresh forage were taken in the field, they were frozen and subsequently the pH was measured in the laboratory. The determination of ASC was carried out in 40–50 mg of sample previously ground using the method of Dubois et al. [9]. The buffer capacity of the fresh forage was determined in 2.5 g fresh samples according to the method described by Jasaitis et al. [10].

### 2.1.2. Variables measured in the silage

The measurements made in the silage comprised the following variables: (i) temperature, pH and ASC content, such as in fresh forage, (ii) aerobic deterioration, (iii) crude protein content (CP) and neutral detergent fiber (NDF), (iv) rate of ruminal fermentation and *in vitro* disappearance of DM, and (v) NH<sub>3</sub>-N content.

At the opening of each silo, the temperature of the silage was measured at five points of the surface layer, and five points at a depth of 30 cm; 100 g samples were taken, which were refrigerated for pH measurement in the laboratory 4 h later. Likewise, samples were taken to which the ASC content was determined with the same procedure used in fresh forage samples.

The aerobic deterioration of the silage was estimated with measurements of temperature and CO<sub>2</sub> production. Three 300 g silage samples were placed in 1 L transparent glass jars. Two of those jars were used to measure CO<sub>2</sub> production following Crossno et al. [11]; the third one remained 5d uncovered and was used to measure the temperature at 08:00, 14:00 and 20:00 h.

The CP content of the silage was estimated by the Kjeldahl method [12], while to estimate its NDF content, the Van Soest method described by 13 Sosa [13] was used.

The ruminal fermentation of silage samples was estimated using the gas production technique [14] with three replicates per sample. The gas pressure generated by the fermentation was measured with a manometer with a scale of 0–1 kg cm<sup>-2</sup>, equipped with a three-phase key and hypodermic needle, the measurements were made with intervals of one-hour in the first 24 h and then every 4 h, after each reading the pressure was made equal to zero. The total gas production was estimated and once the incubation period was over, the residue was filtered and dried, which was considered as the residual DM. The in vitro disappearance of DM (DM<sub>ivD</sub>) was calculated as the difference between initial DM and residual DM.

For the estimation of the NH<sub>3</sub>-N content, metaphosphoric acid was added to the samples and NH<sub>3</sub> was quantified by ultra violet light chromatography with a visible ultraviolet light spectrophotometer (UV/VIS Lambda 35, Perkin Elmer, Waltham, Massachusetts) at 630 nm [15].

Data were analyzed using a generalized linear model (GLM procedure 16 SAS 9.0®) which corresponded to the complete factorial arrangement and hence with effects of cutting schedules, LAB inoculant, wilting times, double interactions, phases and replicates within phases, the Tukey procedure ( $\alpha=0.05$ ) was used for comparisons of LS-means [16]. Additionally, a Principal Component Analysis was carried out with Microsoft Excel® using XLSTAT® (Addinsoft, New York, NY).

### 3. Results

The average pH of the fresh forage was  $6.9 \pm 0.3$  and its buffering capacity was equivalent to  $339 \pm 50$  meq 10<sup>-3</sup> lactic acid, with no effect of the combinations of experimental phases, harvesting hours, wilting times and DM content of the forage at the time of ensiling.

#### 3.1. Dry matter content of forage at cutting and post-wilting

The DM content of forage wilted for 2 h did not differ ( $p < 0.05$ ) from that of the forage wilted for 1 h. The forage cut at 14:00. had a higher DM content than that cut at 08:00 h in Phase 1 ( $p = 0.0029$ ) and in Phase 2 ( $p = 0.0065$ ), but not in Phase 3 ( $p = 0.3348$ ) (**Table 1**). No differences ( $p > 0.05$ ) were found in DM content between wilting 1 or 2 h. The post-wilting DM content was not affected by wilting in phase one; but, in phases two and three the forage after 1 or 2 h of wilting, cut at 2:00 PM had a higher DM content than that cut at 08:00 h under the same wilting time ( $p < 0.05$ ) (**Table 1**).

#### 3.2. Contents of crude protein, neutral detergent fiber and alcohol-soluble carbohydrates in forage to be ensiled

Hour of cutting and wilting time did not affect ( $p > 0.05$ ) CP content in the three experimental phases; contents were on average  $18.5 \pm 1.2$ ,  $16.7 \pm 0.7$  y  $16.0 \pm 0.6$ . In phase one the content of ASC was not affected ( $p > 0.05$ ) by hour of cutting or wilting time, while in phases two and three it was higher ( $p < 0.05$ ) in forage harvested at 14:00 (**Table 2**).

	Phase 1	Phase 2	Phase 3
Hour at cutting	Freshly cut forage DM(%)		
8:00	23 ± 1.1 <sup>b</sup>	17 ± 0.7 <sup>b</sup>	20 ± 1.1 <sup>a</sup>
14:00	29 ± 1.1 <sup>a</sup>	21 ± 0.7 <sup>a</sup>	22 ± 1.1 <sup>a</sup>
	Post-wilting DM (%)		
8:00	27 ± 2 <sup>a</sup>	20 ± 2 <sup>b</sup>	19 ± 1 <sup>b</sup>
14:00	27 ± 2 <sup>a</sup>	24 ± 2 <sup>a</sup>	26 ± 1 <sup>a</sup>

<sup>ab</sup> Means with different literal within columns (of freshly cut or after wilting) are different ( $p \leq 0.05$ ).

**Table 1.** Dry matter content (DM) of alfalfa and orchard grass herbage under two harvest hours, freshly cut and after one or 2 h of wilting during three experimental phases (LS-means).

Hour at cutting	Wilting time (h)	ASC (%)			NDF (%)		
		Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3
8:00	0	3.8 <sup>a</sup>	3.4 <sup>b</sup>	4.3 <sup>b</sup>	57 <sup>d</sup>	75 <sup>a</sup>	69 <sup>b</sup>
8:00	1	3.5 <sup>a</sup>	3.1 <sup>b</sup>	4.0 <sup>b</sup>	63 <sup>b</sup>	75 <sup>a</sup>	73 <sup>a</sup>
8:00	2	3.3 <sup>a</sup>	3.3 <sup>b</sup>	4.3 <sup>b</sup>	68 <sup>a</sup>	75 <sup>a</sup>	66 <sup>c</sup>
14:00	0	4.3 <sup>a</sup>	3.9 <sup>a</sup>	4.7 <sup>a</sup>	60 <sup>c</sup>	73 <sup>b</sup>	67 <sup>bc</sup>
14:00	1	5.1 <sup>a</sup>	4.5 <sup>a</sup>	4.5 <sup>a</sup>	61 <sup>bc</sup>	71 <sup>c</sup>	67 <sup>bc</sup>
14:00	2	3.8 <sup>a</sup>	4.2 <sup>a</sup>	4.9 <sup>a</sup>	57 <sup>d</sup>	71 <sup>c</sup>	63 <sup>d</sup>
SE of means		0.8	0.2	0.2	2.6	1.9	2.1

<sup>abcd</sup> Means that do not share any literal within columns are different ( $p \leq 0.05$ ).

**Table 2.** LS-means and standard error (SE) of alcohol soluble carbohydrates (ASC) and NDF alfalfa and orchard grass forage before ensiling.

### 3.3. Temperature, pH and ammoniacal nitrogen of silages

The average temperature at the opening of the silos was  $21 \pm 0.6^\circ\text{C}$  in phase one,  $20 \pm 0.7^\circ\text{C}$  in phase two and  $18 \pm 1.1^\circ\text{C}$  in phase three, not affected by treatments. In phase one, the silage pH of forage harvested at 14:00 and with inoculant was lower ( $p < 0.05$ ) than that of forage harvested at the same time but without inoculant and those of forage harvested at 08:00 (**Table 3**). The  $\text{NH}_3\text{-N}$  content was not affected ( $p > 0.05$ ) by treatments in phases one and two. In phase three the  $\text{NH}_3\text{-N}$  content of forage harvested at 14:00 and with inoculant was lower ( $p < 0.05$ ) than that of forage harvested at the same time but without inoculant and those of forage harvested at 08:00 (**Table 3**).

### 3.4. Alcohol-soluble carbohydrates in silages

The ASC in freshly cut forage was reduced by ensiling and hence was lower in the correspondent silages, in such a way that there was a linear relationship ( $p < 0.01$ ) between ASC in

Hour at cutting	Inoculant	pH			N-NH <sub>3</sub> (% of total N)		
		Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3
8:00	0	4.7 <sup>b</sup>	4.9 <sup>a</sup>	4.8 <sup>a</sup>	11 <sup>a</sup>	16 <sup>a</sup>	15 <sup>b</sup>
8:00	1	4.8 <sup>a</sup>	4.9 <sup>a</sup>	4.9 <sup>a</sup>	11 <sup>a</sup>	14 <sup>a</sup>	18 <sup>a</sup>
14:00	0	4.8 <sup>a</sup>	4.6 <sup>b</sup>	4.6 <sup>b</sup>	11 <sup>a</sup>	14 <sup>a</sup>	13 <sup>c</sup>
14:00	1	4.4 <sup>c</sup>	4.6 <sup>b</sup>	4.7 <sup>b</sup>	9 <sup>a</sup>	14 <sup>a</sup>	12 <sup>c</sup>

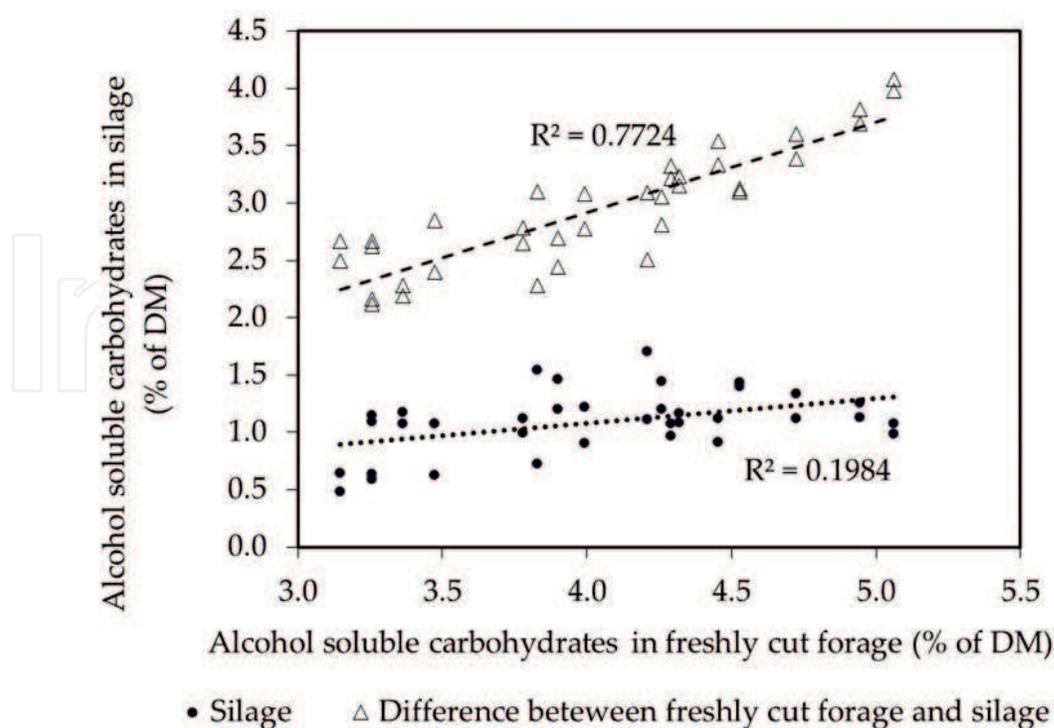
<sup>abc</sup> Means that do not share any literal within columns are different ( $p \leq 0.05$ ).

**Table 3.** Means pH and ammoniacal nitrogen of silages alfalfa and orchard grass cut at two different hours, with or without addition of bacterial inoculant, in three distinct experimental phases.

freshly cut forage and the reduction in content due to ensiling (**Figure 1**). There were significant differences between the treatments in phase two because of the wilting time factor, where the treatments without wilting maintained an ASC of 1.2%, with one-hour of wilting was 0.8% and with 2 h of wilting 1.1%.

### 3.5. Nutritional composition of silages

The CP content of silages was not affected ( $p > 0.05$ ) by the treatments, it was on average 18% in phase one, 15% in phase two and 16% in phase three. The differences in CP of silage with respect to freshly cut forage were small, in phase one the protein of the silage was 0.5% lower, in phase two the decrease was 1.7% while in phase three contents were similar.



**Figure 1.** Relationship between the content of alcohol-soluble carbohydrates in the original forage and its corresponding silage.

In all three phases the NDF content decreased 10% in silage with respect to that of freshly cut forage. In phases one and three the NDF content in the silage was not affected by the treatments ( $p > 0.05$ ). In phase two the hour of cutting x wilting time interaction led to differences ( $p < 0.05$ ); while silage from forage harvested at 8:00 with one-hour wilting and at 14:00 with 2 h wilting had the highest contents, those from forage harvested at 8:00 with 2 h wilting and at 14:00 with one-hour wilting had the lowest contents (Table 4).

### 3.6. Fermentation and ruminal *in vitro* disappearance of DM

The volume of gas produced by *in vitro* silage fermentation was not affected by the treatments in phase one ( $p > 0.05$ ); however, in phase two the volume of gas produced by the fermentation of silage from forage harvested at 08:00 h was lower ( $p < 0.05$ ) than the volume reached with forage harvested at 14:00 (Table 5). In phase three, the gas volumes were affected ( $p < 0.05$ ) by the interaction of wilting time x inoculant; treatments without wilting and with 2 h of wilting with inoculant application reached higher gas volumes than treatments without inoculant. In the case of treatments with one-hour of wilting, the upper value of gas volume was achieved in treatments without inoculant. The *in vitro* disappearance of DM did not show significant differences between treatments of phases one and two; on the other hand, in phase three the percentage of disappearance was lower ( $p < 0.05$ ) in silages from forage harvested at 08:00 h (Table 5).

### 3.7. Aerobic stability of silages

The measurement of the rate of change of temperature did not produce clear results. In phase one the interactions hour at cutting x inoculant ( $P < 0.05$ ) on the rate of change of temperature resulted in silage from forage harvested at 8:00 with one-hour wilting and at 14:00 with 2 h wilting the highest rates were found, while in silages from forage harvested at 8:00 with 2 h wilting and at 14:00 with one-hour wilting the lowest rates were detected. In the same first phase the results of the wilting time x inoculant interaction were of such a nature that no

Hour at cutting	Wilting (h)	NDF (% of DM)		
		Phase 1	Phase 2	Phase 3
8:00	0	47 <sup>a</sup>	59 <sup>b</sup>	63 <sup>a</sup>
8:00	1	48 <sup>a</sup>	61 <sup>a</sup>	64 <sup>a</sup>
8:00	2	45 <sup>a</sup>	54 <sup>e</sup>	60 <sup>a</sup>
14:00	0	48 <sup>a</sup>	57 <sup>c</sup>	62 <sup>a</sup>
14:00	1	50 <sup>a</sup>	56 <sup>d</sup>	65 <sup>a</sup>
14:00	2	51 <sup>a</sup>	61 <sup>a</sup>	59 <sup>a</sup>

<sup>abcde</sup> Means that do not share any literal within columns are different ( $p \leq 0.05$ ).

**Table 4.** Means of NDF content of silage from alfalfa and orchard grass forage harvested at two distinct times of the day and subjected to different times of wilting during three experimental phases.

Hour at cutting	Phase 1	Phase 2	Phase 3
	Gas volume (ml g <sup>-1</sup> DM)		
08:00	218 <sup>a</sup>	193 <sup>b</sup>	227 <sup>a</sup>
14:00	223 <sup>a</sup>	209 <sup>a</sup>	238 <sup>a</sup>
	Ruminal <i>in vitro</i> disappearance of DM (%)		
08:00	60 <sup>a</sup>	49 <sup>a</sup>	50 <sup>b</sup>
14:00	58 <sup>a</sup>	50 <sup>a</sup>	54 <sup>a</sup>

<sup>ab</sup> Means that do not share any literal within columns are different (p ≤ 0.05).

**Table 5.** Means of volume of gas produced by fermentation and ruminal *in vitro* disappearance of DM of silage from alfalfa and orchard forage harvested at contrasting times of the day in three experimental phases.

rational interpretation was feasible. In phases two and three no effect (P > 0.05) of treatments on this variable was detected. Differences among means of phases one, two and three (0.17, -0.27, 0.03°C d<sup>-1</sup>) were also odd.

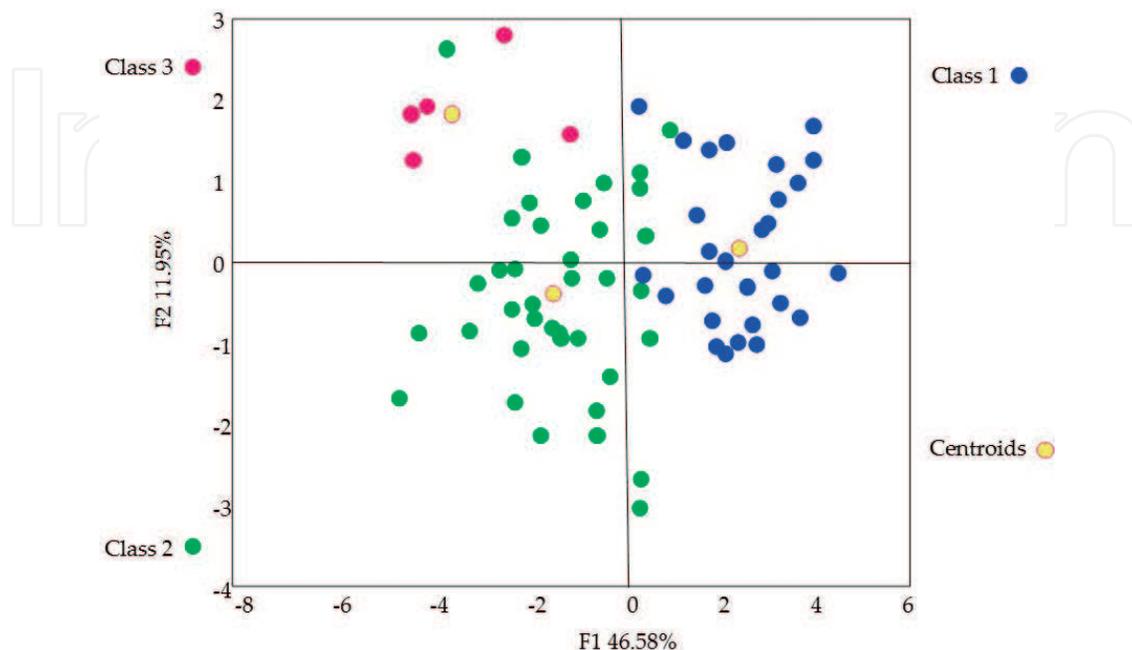
Concerning the variable CO<sub>2</sub> production rate, no effects of treatments were detected in any of the three phases (P > 0.05); the rates of CO<sub>2</sub> production were 9, 9.4 and 19.8 milli moles of CO<sub>2</sub> g<sup>-1</sup> DM d<sup>-1</sup> in phases one, two and three, respectively. In the rate of change of pH, the interaction Hour at cutting x Inoculant (**Table 6**) in the three phases implied that inoculation was not effective to control pH rise in silage from forage cut at 08:00 but it was on silage from forage cut at 14:00 (p < 0.05). The interaction Inoculant x wilting time (**Table 6**) in phase one

		Rate of change of pH units d <sup>-1</sup>		
Hour at cutting	Inoculant	Phase 1	Phase 2	Phase 3
08:00	0	0.18 <sup>d</sup>	0.86 <sup>a</sup>	0.18 <sup>a</sup>
08:00	1	0.63 <sup>a</sup>	0.95 <sup>a</sup>	0.42 <sup>a</sup>
14:00	0	0.37 <sup>b</sup>	0.58 <sup>b</sup>	-0.002 <sup>b</sup>
14:00	1	0.25 <sup>c</sup>	0.32 <sup>b</sup>	0.15 <sup>a</sup>
Inoculant	Wilting time	Phase 1	Phase 2	Phase 3
0	0	0.33 <sup>d</sup>	0.91 <sup>a</sup>	0.12 <sup>a</sup>
0	1	0.10 <sup>e</sup>	0.77 <sup>a</sup>	0.05 <sup>a</sup>
0	2	0.40 <sup>c</sup>	0.49 <sup>a</sup>	0.09 <sup>a</sup>
1	0	0.04 <sup>f</sup>	0.72 <sup>a</sup>	0.30 <sup>a</sup>
1	1	0.84 <sup>a</sup>	0.65 <sup>a</sup>	0.57 <sup>a</sup>
1	2	0.45 <sup>b</sup>	0.52 <sup>a</sup>	0.00 <sup>a</sup>

<sup>abcde</sup> Means that do not share any literal within columns of each interaction are different (p ≤ 0.05).

**Table 6.** Means of rate of change of pH during aerobic deterioration of alfalfa and orchard grass silages.

without inoculant, one-hour wilting precluded the rise of pH, but two-hour wilting did not ( $p < 0.05$ ), while with the addition of inoculant, wilting led to rises in pH ( $p < 0.05$ ), in phases two and three there was no effect of this interaction ( $p > 0.05$ ).



**Figure 2.** Classes of alfalfa and orchard grass silages harvested at 08:00 or 14:00, submitted to 0, 1 or 2 h of wilting and with or without addition of bacterial inoculant.

Class	1	2	3
Harvest at 14:00 proportion (%) within class	89%	26%	17%
Harvest at 08:00 proportion (%) within class	11%	74%	83%
Wilting	75%	58%	83%
Inoculant	46%	50%	67%
Events rainfall	11%	16%	100%
Amount rainfall (mm/6 h)	0.3	0.2	0.7
Evapotranspiration (mm/6 h)	2.5	0.7	0.3
DM % Freshly cut forage	25%	20%	19%
DM % Silage	29%	20%	18%
pH	4.56	4.80	5.18
CO <sub>2</sub> Production rate mmol CO <sub>2</sub> g <sup>-1</sup> DM d <sup>-1</sup>	5.8	17.2	16.2
Temperature °C	20.4	19.1	19.9

**Table 7.** Attributes of three classes of alfalfa and orchard grass silages harvested at 08:00 or 14:00, wilted 0, 1 or 2 h and with or without addition of bacterial inoculant.

### 3.8. Results of principal component analysis

Three classes of silages were identified; Class 1, Class 2 and Class 3 comprising 39%, 53% and 8% of silages (**Figure 2**). Characteristics of those classes are described in **Table 7**.

## 4. Discussion

### 4.1. Properties of forage to be ensiled

The pH of forage to be ensiled was not affected by treatments and ranged between 6.5 y 7.0 in the scope of results quoted by Coblenz and Muck [4], but somewhat higher than the range between 6.1 and 6.2 reported by Santos and Kung [17]. The results attained in terms of buffering capacity with no effect of treatments, differ from those reported by Zheng et al. [18] who found that wilting resulted in an on average reduction in 5% of the buffering capacity of alfalfa forage; but, the effect of wilting on the buffering capacity is highly dependent on the weather conditions during wilting [4].

As expected from results reported by Tremblay et al. [3] DM content of forage cut in the afternoon was higher than that of forage cut in the morning, which was coupled with higher humidity and lower ambient temperature, solar radiation and wind speed in the morning hours, leading to lower evapotranspiration which concurs with Owens et al. [19]. During morning hours low evapotranspiration is the probable cause of the lack of effect of wilting on DM content of forage to be ensiled; conversely, in forage harvested at 14:00 in phases two and three there was a linear increase in DM content as the wilting time increased.

The content of ASC of fresh forage in phase one did not show significant differences, while in phases two and three forage treatments harvested at 14:00 had higher concentrations of ASC, which was to be expected [3]. On the other hand, the wilting time did not affect the concentration of ASC; on the contrary, Zheng et al. [18] found reductions of 8 and 17% when wilting alfalfa forage for 2 and 4 h.

The NDF content of forage harvested at 14:00 was lower than that of forage harvested at 08:00, probably due to the accumulation of photosynthetic products in the cell content [19]. This represents advantages in terms of the nutritional composition and fermentative characteristics of the original forage [3].

### 4.2. Characteristics of silages

Silage temperatures (in the range between 18 and 21°C) were adequate, Borreani and Tabacco [20] report that in well preserved silage the temperature should be close to the ambient temperature.

Silage pH is one of the main factors that influences the degree of proteolysis [21] in silages; the results attained (**Table 3**) were not conclusive since treatments effects differed between phases and short-term weather variables are a feasible explanation for these differences. The pH values fluctuated between 4.6 and 4.9; analogously, in alfalfa silage with *L. buchneri* inoculant

[22] detected a pH of 5.0. In phase two, the pH values of silage from forage harvested at 14:00 were lower, which would coincide with higher levels of MS and ASC contents, conditions that optimize LAB activity and explain the advantage of harvesting forage in the afternoon hours [19]. According to Tyrolová and Výborná [23] for forages with less than 20% DM, it is necessary to acidify the forage up to a minimum pH of 4.2 and for forages with 30% DM a pH of 4.45 is acceptable, which implies that average pH in silages of this experiment were somewhat high. Concurrently, Kung [24] states that pH values higher than 4.6 to 4.8 in legume silages may be due to ensiling at DM contents lower than 30%.

The  $\text{NH}_3\text{-N}$  content is an important indicator, since it shows the amount of protein that has been degraded to ammonia. High  $\text{NH}_3\text{-N}$  contents such as found in this experiment (except for silages from forage harvested at 14:00 and with inoculant added) result from extremely high breakdown of protein, which is frequent in silages with DM contents lower than 30% [24].

The concentration of ASC in the original forage was on average 4.1% and was reduced in all silages in increasing proportion as the initial content was higher ( $P < 0.01$ ), indicating that they were efficiently used by LAB [21]. If there had been a higher concentration of ASC in the original forage, the silage pH would have been even lower; therefore, according to the results of the review by Yitbarek and Tamir [25], the addition of a source of highly fermentable carbohydrates such as molasses would be a suitable alternative.

In the three phases the content of NDF in silage decreased as the wilting time increased, an effect that coincides with the results of Hashemzadeh-Cigari et al. [26] who found that wilting alfalfa before ensiling decreased the NDF content in silage.

The *in vitro* disappearance of DM presented average values of 59, 50 and 52% in phases one, two and three respectively and are lower than the values reported by Rizk et al. [27] who reported an average of 65%, these differences can possibly be attributed to the fact that in the present study the ensiled forage had low DM content.

#### *4.2.1. Aerobic stability of silages*

Forage silages harvested at 08:00 h and inoculated were less stable than those ensiled without inoculants; on the other hand, in forage harvested at 14:00, inoculated silages were more stable. Similarly inoculants were more effective in improving different silage attributes when applied to wilted forage than to fresh forage [26]. Improvement of the aerobic stability characteristics of silages, as in the present study, might be expected with the use of heterofermentative LAB inoculants or mixed heterofermentative and homofermentative LAB [7].

### **4.3. Results of principal component analysis**

In Class 1 the best quality silages were found, with lower pH, lower aerobic deterioration and higher DM content, the silages of that class were, in a very high proportion, harvested at 14:00 with very low proportion of rain events, relatively high evapotranspiration, mostly subjected to wilting (75% versus 66% expected), with highest DM% of original forage, and as in the other two classes no clear effect of inoculant addition. The highest proportion of silages was identified in Class 2, of lower quality than Class 1. The main differences between these

two classes were harvest time (Class 2 mostly harvested at 08:00) and, during the previous 6 h to sealing of silos, 72% lower evapotranspiration and 47% higher proportion of rain events. Class 3 comprised a low proportion of spoiled silage which faced heavy rainfall in the 6 h prior to the closure of silos.

From the above, it follows that the harvest in the afternoon after consulting weather forecasts trying to ensure that there will not be rain events and that there will be conditions for high evapotranspiration are essential factors for the success of ensiling; these results concur with those of Coblenz and Muck [4]. If these conditions are met, wilting and inoculation can contribute to improve the silage quality.

## 5. Conclusions

Harvesting alfalfa and orchard grass forage at 14:00 was advantageous since it led to silage with higher contents of dry matter and alcohol soluble carbohydrates and lower content of neutral detergent fiber.

Absence of rain and high evapotranspiration favored the achievement of positive effects of harvesting in afternoon hours. The effect of wilting and inoculation were bound to rainfall and evapotranspiration conditions during ensiling; under good weather conditions of wilting and inoculation contributed to improve the silage quality in terms of high dry matter content, low pH and better aerobic stability.

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## Conflict of interest

Authors declare they have no conflict of interest.

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