

## ***Conservation of Mulberry as Silage.***

### ***I. Effect on Nitrogenous Compounds***

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#### ***Introduction***

Due to its high edible biomass yield of 16-18 tons DM/ha/year to its high percentages of crude protein (15-25%) and in vitro dry matter (DM) digestibility (75-85%); together with its perennial nature and adaptation to various soil types (CATIE, 1986), mulberry is beginning to extend widely in the livestock regions and is likely to become a forage of excellence for feeding and supplementing ruminants.

Even if it is true that with the use of forage trees the seasonally of production is attenuated, in fact in order to guarantee feeding in the dry periods, it is indispensable that the ratio of production to unit of land be established on the basis of the periods with less yields.

This situation is clear in the case of mulberry, with a cutting interval of three months, there is a surplus in the rainy season. If this additional forage is not harvested, there is an imbalance in the nutritional quality of the shoot due to ageing, a decrease in edible biomass and waste of productive potential (Martín, 1999, unpublished).

One of the ways to avoid this situation is by conserving as silage all the green material non-utilised. However, it is known that tree forages are particular in relation to the conservation technologies established up to now (Vallejo *et al.*, 1994). In fact,

tree forages contain much higher levels of crude protein (Oviedo et al., 1994), but at the same time, this protein degrades during conservation and the animal performance decreases significantly in comparison to fresh forage (González *et al.*, 1997).

Up to now, few studies have been conducted on the conservation of mulberry as silage, and most of them with a pre-determined opening time (Vallejo, 1996), which does not allow to know the dynamics of protein degradation during conservation. In addition, research carried out in temperate forages has shown protein hydrolysis differs with the type of forage, independently whether silage is made with fresh or wilted material (Messman et al., 1993). This explains why only with individual studies of forages species is possible to elucidate the changes of nitrogenous compounds during conservation.

This is why the objective of this research was to conduct an study on the evolution of nitrogenous compounds in mulberry silages and on their transformations during time, taking into account the main indicators and how the inclusion of different doses of conserving agents and pre-wilting interact.

## ***Materials and Methods***

### ***Experiment 1. Fermentation dynamics in mulberry silage.***

Mulberry forage for this experiment was taken from a 3-year old plantation which received a homogenisation cut in May, beginning the rainy season, and a fertiliser dose of 60kg of N/ha. Forage was collected manually after 90d of re-growth, in the month of July. The green material was chopped to 1-2cm and carefully mixed. Double nylon bags, with 3kg capacity, were used as experimental units. Five bags per treatment were filled and sealed within two hours. Treatments were the opening times: 2, 8, 14, 30, 60, 90, 120 and 180 days.

Parameters measured were: DM, determined in an oven with forced ventilation at 70°C for 48h; total crude protein (TCP) determined by the methodology of AOAC (1965); soluble crude protein (SCP) and ammonia from silage juice extracted with a hydraulic press (Dulphy and Demanquilly, 1984).

Results were analysed with multiple regression equations using the Excel statistical analysis.

### ***Experiment 2. Effect of additives and wilting.***

Mulberry utilised in this study was obtained from the same plantation as experiment 1, except that forage was collected in September, 60d after the previous cut and a fertilisation with 60kg of N/ha. The procedure was similar as in experiment 1, but the treatments were the ones presented in Table 1. Bags were opened after 60d.

**Table 1. Treatments for experiment 2.**

<b>Control</b>	<b>Wilting</b>
Final molasses 2%	Formic acid 0.1%
Final molasses 4%	Formic acid 0.2%
Final molasses 6%	Formic acid 0.3%

The experimental design was a complete randomised block and mean differences were determined with Duncan (1955) test.

Due to the complexity of the indicators studied and their interactions, it was decided to weigh them. The method used was that of mean super-indexes as a way to determine treatment differences for weighing purposes. In cases where two super-indexes were similar, the mean of the respective values was taken. The established system for the indicators was set as follows.

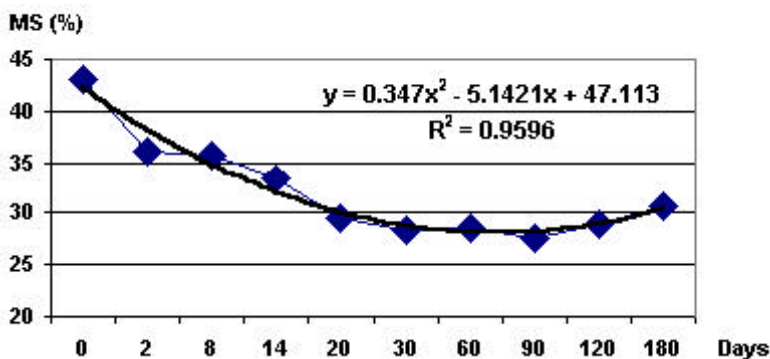
**Table 2.** Weighing of indicators from significant differences expressed by the super-indexes.

DM	TCP	SCP/TCP%	N-NH <sub>3</sub> /N <sub>4</sub> %	pH	Maximum weighing
a-3	a-3	d-3	c-3	c-3	15
b-2	b-2	c-2	2	b-2	
c-1	c-1	b-1	1	a-1	
		a-0		a-1	

## Results

### Experiment 1

Dry matter showed a tendency to decrease during the whole measured period, adjusting well to a quadratic regression (Figure 1). TCP was maintained without major fluctuations (Table 3).

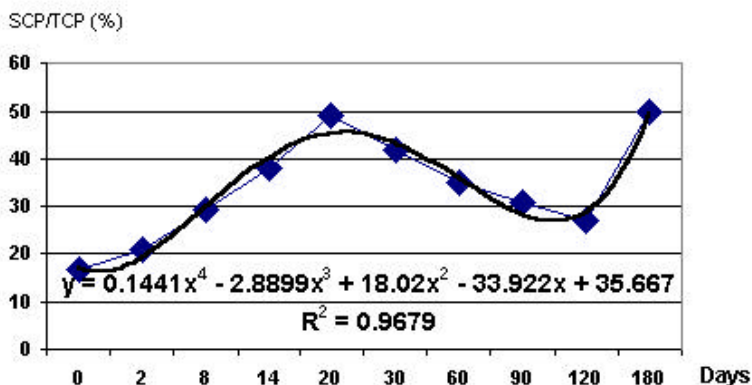


**Figure1.** Dry matter content in mulberry silages with time.

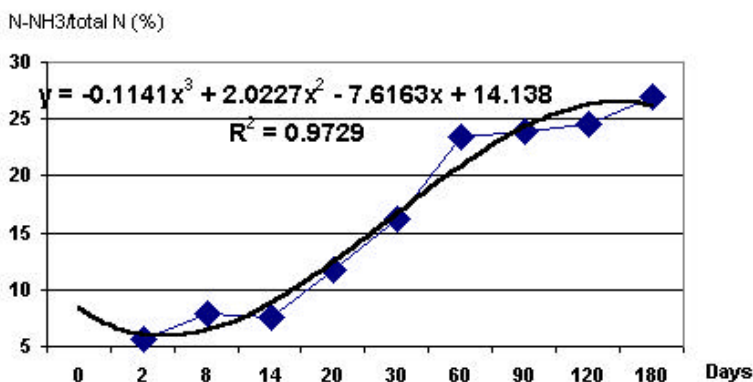
**Table 3.** Changes in total crude protein (%) in mulberry silages.

	Days										SD
	0	2	8	14	20	30	60	90	120	180	
TCP	18.9	18.4	18.4	19.3	18.9	18.0	19.9	18.0	18.5	19.1	4.6

The percent of SPC oscillated but always above the initial value, with marked increases at the end. The best fit was a grade 4 polynomial equation (Figure 2).



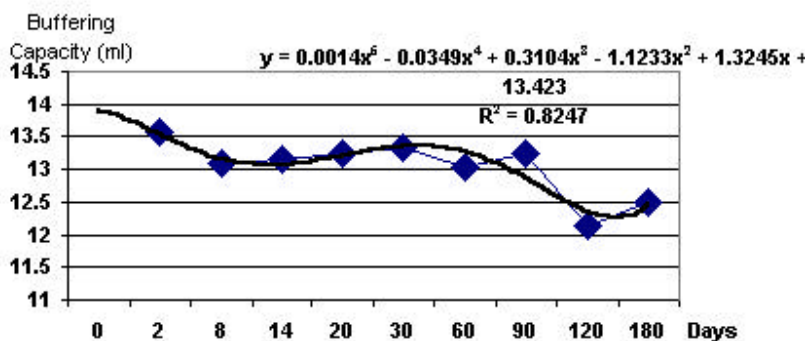
**Figure 2.** Changes in the percentages of soluble protein in mulberry silages.



**Figure 3.** Changes in N-NH<sub>3</sub>/total N [%] in mulberry silages.

The percentage of ammonia N from total N showed an increase from 14d on, with maximum values at 180d. The best fit was a cubic polynomial equation (Figure 3).

The pH dropped rapidly during the first 8d, but at 30d started to rise and the showed a slight decrease at 60d and the a constant value until the end. The best fit was a cubic polynomial but the  $R^2$  values were low (Figure 4).



**Figure 4.** Changes in pH in mulberry silages.

### Experiment 2.

The results of the indicators evaluated are presented in Table 4. The best Dm contents were found in the wilted silages. The addition of final molasses to 4 and 6% of formic acid at 0.2-0.3% produced higher DM contents than the control, but nothing at lower doses. Formic acid favoured better total crude protein conservation the same as wilting with 4% molasses compared to the control. Other treatments were different.

The treatment with the best SCP/TCP ratio was wilting, followed by 2 and 4% molasses and the control. The highest values were those of formic acid.

**Table 4.** Effect of additive and wilting in fermentative quality of mulberry silages.

Treatments	DM	CP	SCP/TCP (%)	pH	NH <sub>3</sub> /total N (%)
Control	31.82 <sup>c</sup>	22.5 <sup>c</sup>	39.9 <sup>c</sup>	5.0 <sup>b</sup>	11.2 <sup>a</sup>
Wilting	40.20 <sup>a</sup>	24.7 <sup>b</sup>	12.3 <sup>d</sup>	5.4 <sup>a</sup>	6.2 <sup>c</sup>
Molasses 2%	33.68 <sup>bc</sup>	21.8 <sup>c</sup>	38.6 <sup>c</sup>	4.9 <sup>b</sup>	12.1 <sup>a</sup>
4%	34.76 <sup>b</sup>	24.2 <sup>b</sup>	38.6 <sup>c</sup>	4.8 <sup>bc</sup>	10.5 <sup>a</sup>
6%	35.67 <sup>b</sup>	23.0 <sup>bc</sup>	43.7 <sup>b</sup>	4.6 <sup>c</sup>	7.5 <sup>bc</sup>
Formic acid: 0.1%	32.67 <sup>c</sup>	26.4 <sup>a</sup>	63.8 <sup>a</sup>	5.0 <sup>b</sup>	9.3 <sup>b</sup>
0.2%	34.67 <sup>b</sup>	27.3 <sup>a</sup>	61.6 <sup>a</sup>	5.0 <sup>b</sup>	13.0 <sup>a</sup>
0.3%	34.60 <sup>b</sup>	26.7 <sup>a</sup>	62.2 <sup>a</sup>	5.3 <sup>a</sup>	14.0 <sup>a</sup>
ES ±	1.36	2.4	0.8	0.1	2.6
Sig %	0.5	0.5	0.1	0.1	0.5

The lowest N-NH<sub>3</sub>/total N were obtained with wilting and with 6% molasses, not different to formic acid at 0.1%. Other treatments, with higher values, were no different.

The lowest pH were obtained with 6 and 4% of molasses. The later no different to 2% molasses, formic acid at 0.1 and 0.2% and control. The highest values were with 0.3% formic acid and wilting.

The analysis of the relative weighing of the results considering the significance index (Table 5), shows that wilted silage reaches 80% of the possible points, followed by 6% molasses, but with a difference of 13 points between them.

Silages with 0.1 and 0.2% of formic acid were of better quality than 0.3% formic acid, and this similar to control silages.

**Table 5.** Weighing of indicators in mulberry silages wilted or with additives.

Treatments	DM (%)	CP (%)	SCP/TCP (%)	pH	N-NH <sub>3</sub> /N <sub>T</sub> %	Total	Weight %
Control	1	1	2	2	1	7	47
Wilting	3	2	3	1	3	12	80
Molasses							
2%	1.5	2	2	2	1	8.5	57
4%	2	2	2	2.5	1	9.5	63
6%	2	1.5	1	3	2.5	10	67
Formic acid							
0.1%	1	3	0	2	2	8	53
0.2%	2	3	0	2	1	8	53
0.3%	2	3	0	1	1	7	47

## ***Discussion***

### ***Experiment 1.***

Dry matter loses were high, approximately 25%. The explanation for this should be looked from two points of view. Firstly, it is known that during oven drying, silages lose volatile components which underestimate DM values, for this reason, values obtained should be taken with caution (Dulphy and Demanquilly, (1981). Secondly, the duration of the trial. In practice, silages are not store for more than 6 months, thus these loses are the extreme high.

Although Vallejo (1995) found similar loses in silages made from tree foliages, the loses with mulberry in his experiment were lower, resulting from the high fermentative quality of the silage. For this reason additives favour DM conservation and in the case of final molasses, there is an addition supply of solids (De la Fuente, 1990).

It was clear that under the conditions that these silages were prepared, that there was a permanent degradation. There were no signs of stabilisation.

Although TCP values were maintained in all treatments of experiment 1, formic acid addition preserves the quality, being this one of the main advantages to use this additive (Keady and Murphy, 1996).

Mulberry proteins also suffer quality changes, with fermentative and nutritional implications. The forage protein hydrolysis is an inherent process in silage making (Ohshima and McDonald, 1978). Fermentation of mulberry in this experiment, without pre-treatments or additives, could not control this process, since pH 4.3, considered the minimum necessary to stop proteases (McDonald *et al.*, 1991), was never reached. The SCP/TCP increased constantly.

The magnitude of the process was reflected in the fluctuations occurring between 30 and 120d, which indicate condensation and re-arranging of soluble N compounds. In this period almost all the soluble N was in the form of ammonia.

Formic acid addition induced high SCP/TCP ratios. The same effect was found by Carpintero *et al.*, (1979), while studying increasing doses of formic and sulphuric acids in temperate grass-legumes mixes, where acidification promoted higher soluble nitrogen. This was seen as a result of a non-enzymatic hydrolysis. However, in the current work, high ammonia percentages were not found.

This contradiction should be interpreted as the result of the microbe predominating in the silage in case, since they are the main responsible for deamination.

In a study conducted by González *et al.*, (1997) with micro-silos of mulberry, there was a direct relationship between pH and lactic acid, which allowed adequate ammonia values in relation to total N.

From these results it can be inferred that formic acid at 0.3% did not control the undesirable fermentations (Luis et al., 1991), agreeing with low quality index.

This is not the situation with wilted silages, in which pH increases are due to a less intensive but higher quality fermentation (Narsh, 1979).

This line of thought agrees with the results obtained with final molasses. Soluble carbohydrate addition facilitates the rise in acidity by promoting more vigorous lactic fermentation (Ojeda, 1993).

### **Experiment 2.**

The above action was detected in silages with 6% molasses. However, the response to the indicators was an increment in the SCP/TCP ratios compared to other doses, confirming that acidification promoted the presence of soluble N compounds but substantially improving ammonia percentages. Vallejo (1995) also found decreases in pH and ammonia % in mulberry silages with 5% molasses. This effect was attributed to a better quality of fermentation with almost double lactic acid concentration when using molasses compared with silages with no additives.

In this study, the most effective treatment was wilting, since it gave the best indicators and ammonia contents. Although Narsh (1979) only found positive aspects of wilted silages, Ojeda et al., (1998) found that during sun drying of mulberry, the leaves lose water faster, and thus, proteases should be rapidly inactivated, restricting their action during fermentation.

Research on temperate forages has shown that there is different behaviour in protein hydrolysis depending on forage type independently of whether there was wilting (Messman et al., 1993).

From the results of this research, it can be concluded that mulberry silages should receive adequate attention not only in relation to initial crude protein content but also to the ways the nitrogen is transformed. Wilting appears as the most adequate technology to reduce protein degradation during conservation.

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