

# Fermentation quality of lucerne laboratory silage treated with *Clostridium cellobioparum*, formic acid and Lactomix

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## ABSTRACT

The experiment was designed to compare and evaluate the effect of the addition of *C. cellobioparum* bacteria, or other standard additives, to lucerne silage noting the resulting quality. The first cut of direct-cut lucerne was ensiled in laboratory scale and treated as follows: I. untreated (control), II. formic acid (FA) 80% – 2 ml kg<sup>-1</sup>, III. *Clostridium cellobioparum* live cellulolytic bacteria culture added in a water suspension at the rate of 10 ml kg (Cclb) – 1x 10<sup>6</sup> g<sup>-1</sup>, IV. *Clostridium cellobioparum* bacteria culture – 1x10<sup>6</sup> g<sup>-1</sup> live cellulolytic bacterial culture added in a water suspension at the rate of 10 ml/kg (Cclb) and Lactomix (LAC) – 1.5 g kg<sup>-1</sup>, V. Lactomix – 1.5 g kg<sup>-1</sup>.

Following an eight-week storage time the results were analyzed. FA treated silage was well preserved, with a pH of 4.10 and protein degradation rate of 75.4 g ammonia N kg<sup>-1</sup> total N. The fermentation parameters of the Cclb inoculated silage were within an acceptable range, with a pH of 4.17 and degradation rate of 102.6 g ammonia N kg<sup>-1</sup> total N. The ammonia levels of LAC inoculated silage were found to be 119.5 g ammonia N kg<sup>-1</sup> total N. Hence, the Cclb was found to have better fermentation quality than the LAC-inoculated samples, but slightly lower than the FA treatment.

KEY WORDS: lucerne silage, additives

## INTRODUCTION

Previous experiments (Baranowski and Russel, 1993) indicate that inoculation of direct-cut meadow grass with cellulolytic bacteria culture *Clostridium cellobioparum* decreases fibre fraction content in silage when compared to untreated silage. The reduction in neutral detergent fibre (NDF) and acid detergent fibre (ADF) content, along with higher amounts of fermentation acids, is thought to occur due to the bacterial degradation of cell wall components and hence promotes the availability of a more fermentable substrates. The present

experiment was designed to evaluate the effect of adding *C. cellobioparum* culture on silage fermentation in relation to other additives.

#### MATERIAL AND METHODS

The silages were obtained from the first cut of lucerne (*Medicago sativa* L.), harvested on 24 June (flowering), and chopped into 1 cm long chaffs. Three samples of original forage ( $n=3$ ) were taken before treating and ensiling, for chemical analysis.

The direct-cut lucerne was ensiled on a laboratory scale (11 Weck jars; ca 750 g fresh forage). Treatments were as follows:

- I. untreated (control),
- II. formic acid (FA) 80% – 2 ml kg<sup>-1</sup>,
- III. *Clostridium cellobioparum* live cellulolytic bacteria culture added in a water suspension at the rate of 10 ml/kg (Cclb) –  $1 \times 10^6$  g<sup>-1</sup>,
- IV. *Clostridium cellobioparum* bacteria culture –  $1 \times 10^6$  g<sup>-1</sup> live cellulolytic bacteria culture added in a water suspension at the rate of 10 ml kg (Cclb) and Lactomix (LAC) – 1.5 g kg<sup>-1</sup>,
- V. Lactomix – 1.5 g kg<sup>-1</sup>.

Each treatment was ensiled in 4 weighed jars, constituting four replicates (except treatment V;  $n=3$ ). The jars were stored for eight weeks in the dark at room temperature and were weighed at seven-day intervals to determine gas losses during fermentation (Berg, 1971). Silages obtained were rapidly frozen at  $-20^{\circ}\text{C}$  until chemical analysis.

The proximate analysis of original forage and silages was conducted according to standard methods (Skulmowski, 1974). Determination of NDF, ADF, and acid detergent lignin (ADL) were made according to Tecator Application Notes (Tecator, Sweden) No. 06/78, 03/78 and 04/78, respectively. Dry matter (DM) content of silage was corrected according to Berg and Weissbach (1976). pH value, organic acids, alcohol, and ammonia contents were determined using Polish Standards (Norma Branżowa, 1974).

The differences among treatments were examined by analysis of variance. The means were compared by the Duncan test and the significance was established at  $P \leq 0.05$  and  $P \leq 0.01$ .

#### RESULTS

All of the used additives had a marked effect on gas losses during fermentation (Fig. 1). As could be expected, the untreated silage (I) showed the highest gas losses, which at the end of storage time reached 9.4% of loaded DM. The lowest gas losses were noticed in silage II, treated with FA (1.9% of DM) and silage III,

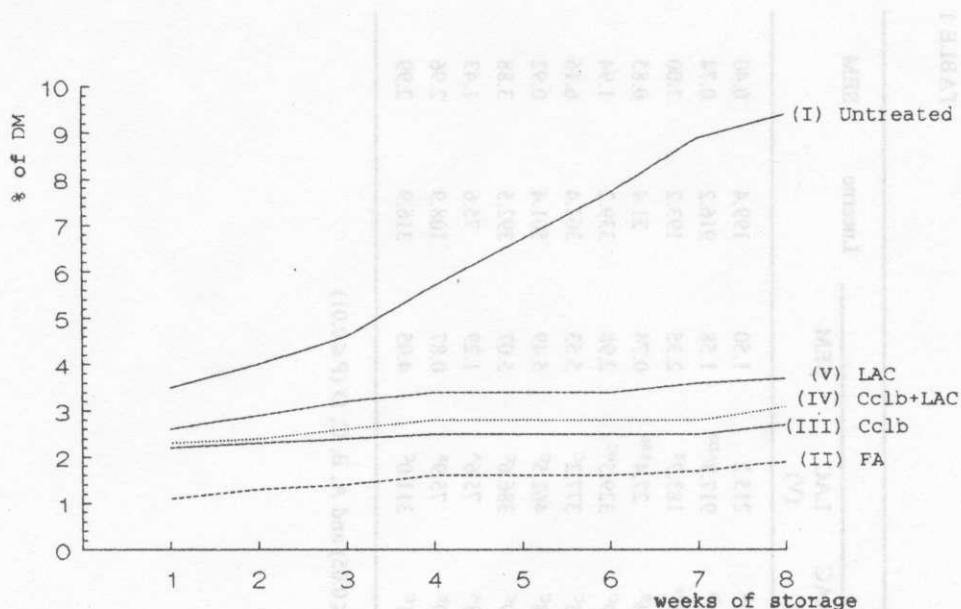


Fig. 1. Gas losses during fermentation of silages (in % of loaded lucerne DM)

inoculated with *C. cellobioparum* (2.7% of DM). Lactic acid bacterial inoculant used alone (V) or together with *C. cellobioparum* (IV) had no effect on the reduction of gas losses (3.7 and 3.1% of DM, respectively) as compared with FA - treated (II) and Cclb-inoculated (III) silages.

The chemical composition of silages and fresh lucerne are given in Table 1. The silages obtained, with additives, were characterized by higher DM content ( $P \leq 0.01$ ) relative to the untreated silage. The differences in the DM content observed among LAC inoculated silage and other treated silages can be partly explained by the different amount of physical nature of the additives used for ensiling lucerne. Marginal differences were observed in the content of crude protein (CP) among Cclb or LAC-inoculated silages and FA-treated silage. However, the untreated silage had a higher CP content than additive-containing silages. The content of NDF and ADF fractions in the untreated silage was significantly higher ( $P \leq 0.01$ ) as compared with all treated silages. The Cclb inoculated silage showed a lower NDF and ADF content than LAC or Cclb with LAC inoculated silages, but the differences were not significant.

The results, shown in Table 2, indicate that additives improved the fermentation parameters of all treated silages in comparison with untreated silage. The pH values of FA treated silage (4.10) and Cclb inoculated silage (4.17) were lower

Composition of lucerne and silages, g kg<sup>-1</sup> dry matter

	Silage treatment					SEM	Lucerne	SEM
	Untreated (II)	FA (II)	Celb (III)	Celb + LAC (IV)	LAC (V)			
Dry matter g, kg <sup>-1</sup>	196.3 <sup>A</sup>	208.3 <sup>Ba</sup>	203.3 <sup>Bb</sup>	207.0 <sup>B</sup>	215.2	1.50	199.4	0.40
Organic matter	914.9 <sup>Aa</sup>	933.2 <sup>B</sup>	923.0 <sup>C</sup>	917.6 <sup>D</sup>	917.8 <sup>ADb</sup>	1.58	916.2	0.74
Crude protein	206.6 <sup>A</sup>	185.0 <sup>B</sup>	187.5 <sup>Ba</sup>	180.5 <sup>Bb</sup>	183.7 <sup>B</sup>	2.35	193.2	3.00
Ether extract	22.9 <sup>Aa</sup>	29.0 <sup>B</sup>	30.1 <sup>B</sup>	29.4 <sup>B</sup>	27.4 <sup>ABb</sup>	0.74	21.4	0.85
Crude fibre	357.7 <sup>A</sup>	324.4 <sup>B</sup>	329.1 <sup>BC</sup>	335.9 <sup>C</sup>	329.5 <sup>BC</sup>	2.98	339.2	1.94
N-free extractives	327.7 <sup>A</sup>	394.8 <sup>B</sup>	376.3 <sup>C</sup>	371.8 <sup>C</sup>	377.2 <sup>C</sup>	5.53	362.4	6.16
NDF	482.7 <sup>A</sup>	415.3 <sup>B</sup>	452.7 <sup>C</sup>	458.8 <sup>C</sup>	462.5 <sup>C</sup>	5.49	501.4	0.92
ADF	409.6 <sup>A</sup>	346.7 <sup>B</sup>	384.6 <sup>C</sup>	384.9 <sup>C</sup>	386.6 <sup>C</sup>	5.07	392.5	3.88
ADL	75.8 <sup>A</sup>	63.2 <sup>B</sup>	75.8 <sup>A</sup>	74.8 <sup>A</sup>	75.6 <sup>A</sup>	1.29	75.6	1.43
Hemicellulose (NDF-ADF)	73.1 <sup>a</sup>	68.6 <sup>Ab</sup>	68.1 <sup>Abc</sup>	73.9 <sup>B</sup>	75.9 <sup>B</sup>	0.87	108.9	2.96
Cellulose (ADF-ADL)	333.8 <sup>A</sup>	283.5 <sup>B</sup>	308.8 <sup>C</sup>	310.1 <sup>C</sup>	311.0 <sup>C</sup>	4.05	316.9	2.99

means in the same line with different letters are significantly different: a, b, c ( $P \leq 0.05$ ) and A, B, C, D ( $P \leq 0.01$ )

TABLE 2

Characteristic of silages

	Silage treatment					SEM
	Untreated (I)	Fa (II)	Cclb (III)	Cclb + LAC (IV)	LAC (V)	
pH	5.67 <sup>A</sup>	4.10 <sup>Ba</sup>	4.17 <sup>B</sup>	4.26 <sup>BCb</sup>	4.36 <sup>C</sup>	0.142
In dry matter, g kg <sup>-1</sup> DM						
lactic acid	28.5 <sup>A</sup>	80.9 <sup>B</sup>	103.4 <sup>Ca</sup>	82.6 <sup>B</sup>	85.0 <sup>BCb</sup>	5.55
acetic acid	34.8 <sup>Aa</sup>	42.2 <sup>Aa</sup>	44.8 <sup>Aa</sup>	77.5 <sup>Ba</sup>	60.5 <sup>b</sup>	4.28
butyric acid	44.7	U	U	U	U	5.55
alcohol	27.9 <sup>Aa</sup>	17.6 <sup>Ba</sup>	23.9 <sup>ACb</sup>	19.2 <sup>BCac</sup>	22.8 <sup>ABbc</sup>	0.99
ammonia	5.9 <sup>A</sup>	2.7 <sup>B</sup>	3.7 <sup>C</sup>	3.9 <sup>DCa</sup>	4.3 <sup>Db</sup>	0.25
Lactic : acetic ratio	0.82	1.92	2.31	1.07	1.40	
In total N, g kg <sup>-1</sup>						
ammonia N	145.3 <sup>A</sup>	75.4 <sup>B</sup>	102.6 <sup>Ca</sup>	111.2 <sup>CDb</sup>	119.5 <sup>D</sup>	5.58

U – undetected

means in the same line with different letters are significantly different: a, b, c ( $P \leq 0.05$ ) and A, B, C, D. ( $P \leq 0.01$ )

than of the remaining samples. Addition of LAC inoculant alone or together with Cclb significantly decreased ( $P \leq 0.01$ ) the pH of experimental silages (4.36 and 4.26 respectively) relative to the untreated one (5.67).

The untreated silage contained less lactic acid ( $P \leq 0.01$ ) than remaining silages. Differences in lactic acid content between FA-treated and LAC or Cclb with LAC inoculated silages (80.9, 85.0, and 82.6 g/kg DM, respectively) were not significant. In contrast, the Cclb inoculated silage showed a significantly ( $P \leq 0.05$  or  $P \leq 0.01$ ) higher lactic acid content (103.4 g/kg DM) than other additive groups. The Cclb inoculated silage had also the lowest acetic acid content (44.8 g/kg DM) as compared with LAC inoculated silage (60.5 g/kg DM;  $P \leq 0.05$ ) or Cclb with LAC (77.5 g/kg DM  $P \leq 0.01$ ). The higher lactic-acetic acid ratio in Cclb inoculated silage than in remaining samples suggests that the use of *Clostridium cellobioparum* leads to a more homolactic fermentation of silage.

The proportion of ammonia-N in total N was significantly higher ( $P \leq 0.01$ ) in untreated silage (145.3 g/kg) as compared to the rest. The LAC inoculated used alone or together with Cclb did not reduce the proportion of ammonia-N in total N in either silages (119.5 and 111.2 g/kg, respectively) as compared with Cclb-inoculated (102.6 g/kg) and FA treated (75.4 g/kg) samples.

## DISCUSSION

As was also shown in previous experiments on grass silage (Baranowski and Russel, 1993), *Clostridium cellobioparum* bacterial additive decreased ( $P \leq 0.01$ ) the NDF and ADF contents in direct-cut lucerne silage compared to the untreated sample. The observed lowest content of fibre fractions in FA treated silage may reflect a relatively strong effect of formic acid on the cell wall components (Morrison, 1979; Jaakkola et al., 1991) The positive effect of formic acid on preservation is commonly known and was here confirmed. The FA treated silage was well preserved, as evidenced by the low pH (4.10) and low protein degradation rate (75.4 g ammonia-N kg<sup>-1</sup> total N).

The addition of commercial lactic acid inoculant (LAC) improved ensiling conditions of fresh lucerne, but fermentation characteristics of the obtained silage were not satisfactory as compared with FA treated and Cclb inoculated silages. The fermentation parameters of Cclb with LAC-inoculated silage (Cclb + LAC) were midway between the two tested individually.

In comparison with lactic acid inoculant, and mixed inoculant, the use of *C. cellobioparum* bacteria inhibited acetic fermentation, supported lactic fermentation, and restricted proteolysis in the silage to the level characteristic for well preserved lucerne laboratory silages (Voss, 1967).

The fermentation using Cclb was accompanied by low gas losses, and was found to be in the range of values given by Rahn (1992) for butyric-free clover and lucerne silages.

Overall, the fermentation parameters of Cclb inoculated silage were acceptable because of the low pH (4.17), high amount of lactic acid (103.4 g/kg DM), and the proportion of ammonia (102.6 g kg<sup>-1</sup> total N), which was higher compared with FA treated silage, but significantly lower ( $P \leq 0.01$ ) in relation to LAC-inoculated silage (119.5 g kg<sup>-1</sup> total N). The fermentation quality of *C. celllobioparum* - inoculated silage was better than that of LAC- inoculated silage and slightly lower than FA-treated silage.

## REFERENCES

- Baranowski A., Russel S., 1993. Quality of meadow grass silage inoculated with cellulolytic bacteria. *J. Anim. Feed Sci.* 2, 59-65
- Berg K., 1971. Die Trockensubstanzbestimmung von Silagen und die Erfassung flüchtiger den Futterwert beeinflussender Verbindungen sowie Modellversuche zur Ermittlung des Gärverlustes. Dissertation. Universität Rostock
- Berg K., Weissbach F., 1976. Untersuchungen zur vollständigen Erfassung des Trockensubstanzgehaltes von Silagen. 1. Mitteilung. Ermittlung der Stoffverluste bei der Trocknung von Silageproben. *Arch. Tierernähr.* 26, 661-672
- Jaakkola S., Huhtanen P., Hissa K., 1991. The effect of cell wall degrading enzymes or formic acid on fermentation quality and on digestion of grass silage by cattle. *Grass Forage Sci.* 46, 75-87
- Morrison I.M., 1979. Changes in the cell wall components of laboratory silages and the effect of various additives on these changes. *J. Agric. Sci., Camb.* 93, 581-586
- Norma Branżowa, 1974. Pasze. Metody oceny jakości i wartości pokarmowej kiszzonek. BN-74/9162-01
- Rahn S., 1992. Untersuchungen zum Einfluss chemischer Siliermittel auf den Gärungsverlauf, die Gärverluste und die aerobe Stabilität von Grünfuttersilagen. Dissertation. Humboldt-Universität zu Berlin
- Skulmowski J., 1974. Metody określania składu pasz i ich jakości. PWRiL, Warszawa
- Voss N., 1967. Untersuchungen über den Proteinabbau in Gras- und Luzernesilage. *Wirt. Futter* 13, 130-145

## STRESZCZENIE

**Jakość kiszonek z lucerny sporządzonych z dodatkiem *Clostridium cellobioparum*, kwasu mrówkowego i Lactomixu**

Pierwszy pokos lucerny (19,94% s.m.) zakiszano w słojach Wecka (ok. 750g świeżej masy) bez dodatków (I) lub z dodatkami: kwasu mrówkowego – 2 ml kg<sup>-1</sup> (II), cellulolitycznych bakterii *Clostridium cellobioparum* – 1x10<sup>6</sup> g<sup>-1</sup> (III), *Clostridium cellobioparum* – 1x10<sup>6</sup> g<sup>-1</sup> i Lactomixu – 1,5 g kg<sup>-1</sup> (IV) oraz Lactomixu – 1,5 g kg<sup>-1</sup> (V).

Po 8-tygodniowym okresie fermentacji pH, zawartość kwasu mlekowego (g kg<sup>-1</sup> s.m.) i amoniaku (g kg<sup>-1</sup> s.m.) oraz udział N-NH<sub>3</sub> w N całkowitym (g kg<sup>-1</sup>) badanych kiszonek wynosiły odpowiednio: (I) – 5,67; 28,5; 5,9 i 145,3; (II) – 4,10; 80,9; 2,7 i 75,4; (III) – 4,17; 103,4; 3,7 i 102,6; (IV) – 4,26; 82,6; 3,9 i 111,2; (V) – 4,36; 85,0; 4,3 i 119,5.

Dodatek bakterii *Clostridium cellobioparum* korzystnie wpłynął na przebieg procesów fermentacyjnych w zakiszanej lucernie niż Lactomixu. Stąd kiszonka sporządzona z dodatkiem bakterii była lepszej jakości niż z dodatkiem Lactomixu, lecz nieznacznie gorsza niż kiszonka z dodatkiem kwasu mrówkowego.