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Quality of meadow grass silage inoculated with cellulolytic bacteria

A. Baranowski[1] and S. Russel[2]

[1] *Institute of Genetics and Animal Breeding, Polish Academy of Sciences Jastrzębiec, 05-551 Mroków, Poland*
[2] *Warsaw Agricultural University (SGGW) Rakowiecka 26/28, 02-528 Warsaw, Poland*

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ABSTRACT

Firest cut of fresh meadow grass (17.3% DM) containing (on DM basis) 13.4% CP, 16.4% WSC, 26.0% CF was ensiled in 1 liter Weck jars without additives (I) or with two strains of live cellulolytic bacteria: *Cellulomonas flavigena* (Cfl) 10^6/g herbage (II), *Clostridium cellobioparum* (Cclb) 10^6/g herbage (III), Cfl 10^3 + Cclb 10^3/g herbage (IV) and Cfl 10^6 + Cclb 10^6/g herbage (V). After 8 weeks of storage, the pH value, DM (%), lactic acid (%) and ammonia-N as a percentage of total silage N were: I - 4.72; 15.3; 1.25 and 12.8; II - 4.42; 16.7; 1.64 and 10.8; III - 3.90; 16.5; 2.01 and 5.3; IV - 3.93; 16.6; 2.13 and 7.3; V - 4.24; 16.7; 1.89 and 7.4, respectively. The amount of NDF and ADF was lower ($P < 0.01$) in Cclb-inoculated silages than in control silage. Laboratory evaluation of the silages revealed a positive effect of addition of Cclb on fermentation and silage quality.

KEY WORDS: silage, cellulolytic bacteria, meadow grass

INTRODUCTION

The preparation of good quality silages from plants containing high levels of protein and low concentrations of easily fermenting carbohydrates requires the use of chemical conservants or additives stimulating lactic acid fermentation (Gross and Beck, 1969; Gross, 1987; Knabe et al., 1987).

Problems related to environmental protection and high quality standard set before products of animal origin limit the application of chemical conservants and give a preference to biological additives. The results of investigations indicate that additives based on lactic acid bacteria do not ensure butyric acid-free silages when ensiling lucerne, clover (Doroszewski and Podkówka, 1989; Doroszewski, 1989) or meadow grass containing less than 30% dry matter (Honig, 1967; Gross, 1987). Obtaining high quality silages from such materials requires wilting the original herbage or adding easily fermenting sugars (Gross and Becl, 1969;

Honig, 1967; Gross, 1987). It is, however, possible to obtain well-preserved silages even from high moisture grass (Jaakkola et al., 1990; Jaakkola and Huhtanen, 1990; Jaakkola et al., 1991) by adding plant cell wall degrading enzymes which improve conditions for lactic acid fermentation (Letherwood et al., 1963; Jacobs and McAllan, 1991; Jacobs et al., 1991). There is no information about the use of cellulolytic bacteria as a source of cellulolytic enzymes.

The aim of the present work was to examine the value of cellulolytic bacteria as an additive to fresh meadow grass silage.

MATERIAL AND METHODS

The silage was made from the first-crop of meadow grass (dominating species *Dactylis glomerata*) harvested on 27 May 1991 and cut into 1 cm long chaff. Three samples of herbage were taken before ensiling for chemical analysis.

Two live strains of mezophile cellulolytic bacteria were used as inoculants: aerobic *Cellulomonas flavigena* (Cfl) and anaerobic *Clostridium cellobioparum* (Cclb), both supplied by the Departament of Agricultural Microbiology, Warsaw Agricultural University. The fresh herbage was ensiled in 5 variations. The bacteria strains and counts are presented in Table 1. The original cultures of live bacteria were diluted to form a water suspension and added at the rate of 1 ml per 100 g of fresh herbage. The material was mixed thoroughly and then loaded and carefully pressed in 1 liter Weck jars. Each treatment was ensiled in 4 jars, constituing 4 replicates (Table 1). The jars were kept for 8 weeks at room temperature. The obtained silages were frozen at -20°C until chemical analysis.

The proximate analysis of herbage and silage was conducted using standard methods (Skulmowski, 1974). Determination of neutral detergent fibre (NDF),

TABLE 1

Experimental design

Silage obtained[1]	Treatments	Counts of live bacteria per g of fresh herbage[2]
I	No additive	
II	*Cellulomonas flavigena*[3] (Cfl)	10^6
III	*Clostridium cellobioparum*[4] (Cclb)	10^6
IV	*Cellulomonas flavigena* (Cfl) + + *Clostridium cellobioparum* (Cclb)	$10^3 + 10^3$
V	*Cellulomonas flavigena* (Cfl) + + *Clostridium cellobioparum* (Cclb)	$10^6 + 10^6$

[1] Four Weck jars per each treatment

[2] Theoretical

[3,4] Isolated from soil and identified according to Bergey's Manual of Determinative Bacteriology, 8 ed. Ed.: R. E. Buchanan and N. E. Gibbons. Baltimore 1974, Williams and Wilkins

acid detergent fibre (ADF) and acid detergent lignin (ADL) were made according to Tecator Aplication Notes Tecator, Sweden Nr 06/78; 03/78 and 04/78, respectively. Water soluble carbohydrates (WSC) were analysed in dry herbage by the method of Haselmore and Roughan (1976). Dry matter (DM) content of silages was corrected according to Berg and Weissbach (1976). Organic acid, alcohol and ammonia contents in silages were determined using Polish Standards (Norma Branżowa, 1974). The silage quality was evaluated by the Flieg-Zimmer index (Skulmowski, 1974).

Differences between treatments were examined by a one-way analysis of variance. The means were compared by Duncan's test and the significances were determined at $P \leqslant 0.05$ and $P \leqslant 0.001$.

RESULTS AND DICSUSSION

The chemical composition of herbage prior to ensiling is presented in Table 2. The herbage contained (on DM basis) 16.4% WSC and 13.4% CP (WSC:CP = = 1.2) and was suitable for ensiling (Podkówka, 1962; Honig, 1967). However, the low DM content (17.3%) could lower the chance of obtaining a silage without clostridial fermentation (Honig, 1967; Renner, 1967, 1968).

TABLE 2

Chemical composition of herbage prior to ensiling (on DM basis)

n = 3	Mean	SD
Dry matter	17.3	0.12
Ash	7.9	0.15
Organic matter	92.1	0.15
Crude protein	13.4	0.33
Ether extract	3.1	0.30
Crude fibre	26.0	0.07
N-free extractives	49.6	0.08
Water soluble carbohydrates	16.4	0.32
Neutral detergent fibre	51.7	0.44
Acid detergent fibre	30.1	0.76
Acid detergent lignin	2.1	0.15
Hemicellulose[1]	21.6	1.36
Cellulose[2]	28.0	0.98

[1] NDF-ADF, [2] ADF-ADL

The DM content (Table 3) indicates the superiority of silages inoculated with anaerobic bacteria, Cclb, given alone or with Cfl, compared with either control silage (I), characterised by the lowest DM content (15.3%) or silage II (10^6 Cfl cells/g fresh herbage) containing 16.0% DM ($P < 0{,}01$). In the two remaining

TABLE 3

Chemical composition of silages, % (on DM basis)

Silage	Dry matter	Ash	Organic matter	Crude protein	Ether extract	Crude fibre	N-free extractives
I mean	15.3^{ABC}	9.1^{Aab}	90.9^{Aab}	15.4^{ABa}	4.9	31.8^{AB}	38.8^{ABC}
II mean	16.0^{ABC}	9.5^{BCa}	90.5^{BCa}	14.5^{a}	4.8	29.6^{AB}	41.6^{AB}
III mean	16.5^{A}	8.2^{ABc}	91.8^{ABc}	14.9	4.8	28.2^{Aa}	43.9^{A}
IV mean	16.6^{B}	8.8^{B}	91.2^{B}	14.5^{A}	4.6	28.5^{B}	43.6^{Ba}
V mean	16.7^{C}	8.6^{Cbc}	91.4^{Cbc}	14.4^{B}	4.6	27.4^{Ba}	45.0^{Ca}
SEM	0.09	0.08	0.08	0.12	0.06	0.27	0.38

Means within each column with the same superscripts differ significantly: A, B, C at $P \leqslant 0.01$ and a, b, c at $P \leqslant 0.05$.

TABLE 4

Silage fibre fractions, % (on DM basis)

Silage	Neutral detergent fibre	Acid detergent fibre	Acid detergent lignin	Hemicellulose[1]	Cellulose[2]
I mean	52.8^{AB}	34.1^{ABa}	2.5^{ab}	18.7^{A}	31.6^{ABC}
II mean	52.4^{CD}	33.2^{CDa}	2.2	19.2^{Ba}	31.0^{DEF}
III mean	48.3^{BC}	30.9^{ADb}	2.1^{a}	17.4^{ABCb}	28.8^{ADa}
IV mean	50.3^{AC}	31.7^{BC}	2.2	18.6^{C}	29.5^{BE}
V mean	48.2^{AD}	29.9^{BCb}	2.1^{b}	18.3^{ab}	27.8^{BFa}
SEM	0.48	0.40	0.06	0.16	0.34

Means within each column with the same superscripts differ significantly: A, B, C, d at $P \leqslant 0.01$ and a, b at $P \leqslant 0.05$

[1] NDF-ADF

[2] ADF-ADL

TABLE 5

Silage quality

Silage	pH	Alcohol %	NH_3 %	NH_3-N in total N %	Lactic acid %	Acetic acid %	Butyric acid %	Flieg index score	
I mean	4.72^{ABC}	0.61	0.058^{ABC}	12.8^{AB}	1.25^{ABC}	0.48^{a}	0.61^{Aa}	45	"moderate"
II mean	4.42^{ABC}	0.51	0.049^{ABC}	10.8^{AB}	1.64^{ABa}	0.53	0.48^{Ba}	60	"moderate"
III mean	3.90^{A}	0.55	0.026^{ABC}	5.3^{AB}	2.01^{A}	0.70	U[1]	88	"very good"
IV mean	3.93^{B}	0.58	0.034^{B}	7.3^{A}	2.13^{Bb}	0.61	0.02^{AB}	88	"very good"
V mean	4.24^{ABC}	0.60	0.034^{C}	7.4^{B}	1.89^{ab}	0.79^{a}	U[1]	88	"very good"
SEM	0.046	0.015	0.0019	0.43	0.059	0.032	0.081		

Means within each column with the same superscripts differ significantly: A, B, C at $P \leqslant 0.01$ and a, b at $P \leqslant 0.05$

[1] Undetectable

silages, which contained a mixed Cfl + Cclb inoculate, the DM level was similar and did not differ significantly from the content of silage III (16.5%). Moreover, compared with silages I and II, silages made with Cclb had a lower CF content ($P < 0.01$). The lower level of CF combined with the significant increase in N-free extractives in the silage obtained with a cellulolytic bacteria additive, especially with the Cclb ($P < 0.01$), could result from partial degradation of structural carbohydrates. This is confirmed by results of fibre fraction analyses (Table 4) which indicate a high cellulolytic activity of anaerobic Cclb bacteria. Its addition to silage III, IV and V resulted in a significant decrease in NDF, ADF and cellulose contents ($P < 0.01$) compared with control and Cfl silages. Moreover, in silage II, made with Cfl, the level of these fractions did not differ significantly ($P > 0.05$) from that observed in control silage. In silage III (10^6 Cclb/g fresh herbage) the content of cellulose, the substrate for cellulolytic enzymes, decreased in comparison with the control silage by 2.80 percent units. According to Jaakkola et al. (1991) and Stokes (1992) the difference between a control grass silage and silages made with the addition of cell wall degrading enzymes used alone or together with lactic acid bacteria, remained within the range of 0.21–1.80 percent units. The degree of structural carbohydrate degradation in silages made with Cclb may also reflect the relatively high cellulolytic activity of *Clostridium cellobioparum* bacteria.

The control and silage II, made with aerobic Cfl had unfavourably high pH values (4.72 and 4.42 respectively) (Table 5). The addition anaerobic Cclb, which degrade structural carbohydrates, also resulted in a decrease in silage pH to 3.90, commonly accepted as satisfactory (Honig, 1967; Renner, 1967; Gross, 1987) and comparable with (3.84–3.95) enzyme-treated silages (Jaakkola et al., 1991). A similar pH value (3.93) was obtained in silage IV, made with an addition of both Cfl and Cclb at 10^3/g of fresh herbage. In turn, the addition of both Cfl and Cclb at the rate of 10^6/g increased the pH to 4.24. This suggests poorer fermentation which probably can be associated with the presence of aerobic Cfl.

The possitive effect of anaerobic cellulolytic bacteria on silage quality was also confirmed by the lower level of ammonia-N as a percentage of total-N which equalled 12.3 and 10.8 respectively, indicating intense protein degradation (Podkówka, 1962; Honig, 1967; Voss, 1967; Gross, 1987). The lowest ($P < 0.01$) protein degradation was observed in silage III (10^6 Cclb/g fresh herbage) — 5.3 of NH_3-N% total N. The share of ammonia-N in all of the silages made with Cclb did not exceed 8%, considered a limit value for good quality silages (Honig, 1967; Voss, 1967; Renner, 1968) and was lower than determined for enzyme-treated grass silages (Jaakkola et al., 1990; Jaakkola et al., 1991).

In the control silage a high level of butyric (0.61%) and a low level of lactic acids (1.25%) were observed. A similar concentration of organic acids, also indicative of an unsatisfactory fermentation process, was found in silage II made

with Cfl. The addition of anaerobic Cclb probably led to an increase in the amount of substrate for lactic acid bacteria. In silage III, made with Cclb, no butyric acid was found and the content of lactic acid (2.01%) was significantly higher ($P<0.01$) compared with the control and silage II. No significant differences were observed in the content of fermentation acids between Cclb inoculated silages. According to the Flieg index, the quality of control and silage II was classified as „moderate". The silages made with Cclb were classed as „very good" and suggested intensive lactic acid fermentation (Podkówka, 1962; Honig, 1967; Renner, 1967; Voss, 1967) also characteristic of well preserved grass silages treated with cell wall degrading enyzmes (Jaakkola et al., 1990; Jaakkola and Huhtannen, 1990; Jaakkola et al., 1991).

The results of the present pilot experiment indicate that:

1. Incoulation by *Cellulomonas flavigena* had a marginal effect on fermentation parameters in silages. Insufficient oxygen levels in silos can probably limit the development of aerobic bacteria and their cellulolytic activity.
2. Addition of anaerobic *Clostridium cellobioparum* stimulated lactic acid fermentation, improving silage quality. The cellulolytic activity of Cclb, confirmed by the analyses of fibre fractions, probably increased the WSC content and improved fermentation conditions.

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STRESZCZENIE

Wpływ dodatku bakterii celulolitycznych na jakość kiszonek z runi łąkowej

Pierwszy pokos runi łąkowej (17,3% suchej masy) zawierającej w suchej masie 13,4% białka surowego, 16,4% cukrów i 26,0% włókna surowego zakiszano w 1-litrowych słojach Wecka bez dodatków (I) lub z dodatkiem dwóch szczepów bakterii celulolitycznych: *Cellulomonas flavigena* (Cfl) 10^6/g zielonki (II), *Clostriduim cellobioparum* (Cclb) 10^6/g zielonki (III) oraz Cfl 10^3 + Cclb 10^3/g zielonki (IV) i Cfl 10^6 + Cclb 10^6/g zielonki (V). Proces fermentacji przebiegał w pokojowej temperaturze i trwał 8 tygodni.

Wartość pH, zawartość (%) suchej masy, kwasu mlekowego oraz procentowy udział azotu amoniakalnego w ogólnym azocie analizowanych kiszonek wynosiły odpowiednio: I — 4,72; 15,3; 1,25 i 12,8; II — 44,42; 16,0, 1,64 i 10,8; III — 3,90; 16,5; 2,01 i 5,3; IV — 3,93; 16,6; 2,13 i 7,3 oraz V — 4,24; 16,7; 1,89 i 7,4. W kiszonkach sporządzonych z dodatkiem Cclb stwierdzono istotnie niższą ($P < 0,01$) zawartość NDF i ADF aniżeli w kiszonce kontrolnej. W porównaniu z kiszonką kontrolną ocenioną w skali Fliega jako „średnia", dodatek bakterii Cclb powodował znaczną poprawę jakości kiszonek ocenionych jako „bardzo dobre".