EFFECT OF BIOLOGICAL ADDITIVE ON FERMENTATION AND NUTRITIVE VALUE OF RED CLOVER-TIMOTHY SILAGE

H. Kaldmäe[1], A. Olt[1], M. Ots[1], O. Kärt[1], E. Songisepp[2]

[1] Estonian University of Life Sciences, [2] Bio-Competence Centre of Healthy Dairy Products

ABSTRACT. *Due to the low fermentation coefficient (27.5) of red clover-timothy mixture, it is necessary to use additives in order to make high-quality silage from it.*

The objective of the study was to select a proper biological additive for ensiling red clover-timothy mixture. DM losses during silage fermentation, organic matter digestibility and fermentation quality were studied.

Test silages were ensiled in 3-litre jars. Four different biological silage additives I-1, I-2, I-3, I-4, and chemical additive AIV 2000 were used.

The use of biological or chemical additives decreased silage DM losses by 2.1 to 3.1 times. DM losses were the lowest (7.7%) when additive I-3 was used.

Treatment with biological additive with lactic acid bacteria concentration 8×10^9 cfu/g significantly improved the fermentation quality of clover-timothy silage: pH 4.0–4.1, ammonia-N in TN 1.1–2.0%, lactic acid content 43–71 g/kg and butyric acid content 0–3 g/kg in DM.

Organic matter digestibility of the treated silage improved up to 10% as compared to that of the untreated silage (P<0.0001).

All studied biological additives improved ensilability of the red clover-timothy mixture with low DM content. Biological additive I-3 appeared to be the most suitable for treating that kind of silage material.

Keywords: biological additive, silage, red clover, quality of fermentation.

Introduction

In recent years much attention has been paid to the growing of legumes and producing legume silage for dairy cattle. Legumes are difficult to ensile because of their high buffering capacity, low contents of dry matter and carbohydrates (McDonald *et al.*, 1991). On the other hand, considering dry matter yield and cultivation expenses, legumes are still cheaper to be cultivated than grasses; however, the cheapest silage has been made from red clover. The cost of legume-grass mixture silage appeared to be cheap as well (Doyle, Topp, 2002).

The quality of silage does not depend only on the chemical composition of grasses or the production arrangement, but also on fermentation, especially on the activity of lactic acid bacteria. The latter is variable, depending on specific properties of strains. In order to use directed lactic acid fermentation, silage material is treated with a selected bacterial starter culture. A specific microbial additive in proper conditions improves the ensilability of fresh material and increases the productivity of animals as shown by Harman *et al.* (1999) and Jatkauskas *et al.*, 2002. The use of an additive has decreased losses and improved the nutritive value of silage (Ruser, Rutherford, 1999; Muck, Shinners, 2001).

The object of the study was to select the most effective biological additive for ensiling red clover and timothy mixture. DM losses during silage fermentation, organic matter digestibility and fermentation quality were studied.

Material and methods

The mixture (1:1) of red clover (*Trifolium pratense* L., variety Jõgeva 433) and timothy (*Phleum pratense* L., variety Tika) was studied. The material was chopped into 2 cm pieces, mixed and conserved in 3-litre jars with three repetitions. For ensiling, chemical additive AIV 2000 and four different biological additives were used. The biological additives were combined from three different strains of *Lactobacillus sp.* as follows:

I-1   *L. plantarum* MTD/1,
I-2   *L. plantarum* MTD/1 + *L. fermentum* KOK5,
I-3   *L. plantarum* 68-4 + *L. fermentum* KOK5 and
I-4   *L. plantarum* 68-4.

Both additives were applied 5 litres per ton of fresh material. The concentration of lactic acid bacteria in the biological additive was 8×10^9 cfu/g.

In 90 days the jars were opened for analysis. For estimating DM losses, DM concentration was determined prior to and after ensiling.

In order to determine the content of volatile fatty acids, ethanol and ammonia nitrogen, and the value of pH, water solution of a silage sample was prepared: 50 g silage was weighed, 100 ml distilled water added and filtrated through a paper filter in 15 hours.

The pH value was measured with a MP 120 Mettler Toledo pH meter, ammonia nitrogen was determined, using an adjusted Kjeldec Auto 1030 Tecator analyser. The contents of ethanol, lactic acid and volatile fatty acids were determined chromatographically, using a Perkin Elmer 900 gas-chromatograph with a column packed with 80/120 Carbopack B-DA/4% carbowax 20 M (Faithfull, 2002).

Samples were dried for 20 hours at 60 °C, chopped into 1 mm-diameter-particles and analysed for the content of DM, crude protein, crude ash and crude fibre (AOAC, 1990). For determining crude ash concentration, samples were reduced to ashes in a furnace at 550 °C for 6 hours. Crude protein was analysed by Kjeldahl method with Kjeldec Auto 1030 analyser (FOSS Tecator, Höganäs, Sweden). The samples were analysed for *in vitro* organic matter digestibility (IVOMD) by the filter bag method, using a DAISY II incubator, fibre analyser equipment (ANKOM Technology, Fairport, NY USA) and reducing to ashes in a furnace. The concentration of NDF and ADF in the samples was determined with a fibre analyser ANKOM 200 (Van Soest *et al*., 1991).

Fermentability coefficient (FC) was calculated according to Pahlow and Weissbach (1999):

$$FC = DM\ [\%] + 8\ WSC/BC, \text{ where}$$

DM – dry matter content %, WSC – content of water soluble carbohydrates, and BC – buffering capacity.

WSC was determined by the anthrone method of Thomas (1977) and BC by titration of lactic acid to pH 4.0.

Statistical analysis

Data were analysed by using GLM procedure of SAS. The effects of treatment and additive were tested by means of orthogonal contrasts. For analysing the traits containing zero values, ranks of values were used; other traits were transformed to their logarithmic values.

The contrasts were calculated from the following model:

$$Y_{ijk} = \mu + T_i + K_j + E_{ijk}, \text{ where}$$

Y_{ikj} – trait; μ – mean; T_i – treatment; K_j – effect of additive; E_{ijk} – random error.

Results and discussion

The mixture of red clover and timothy (1:1) with DM content 185 g/kg was studied. The content of crude protein, crude ash, crude fat, crude fibre, NDF, ADF, N-free extractives and water soluble carbohydrates was 147 g/kg, 82 g/kg, 32 g/kg, 201 g/kg, 432 g/kg, 245 g/kg, 537 g/kg and 101 g/kg in DM, respectively. The buffering capacity of the material was 90 g lactic acid per kg DM and fermentation coefficient 27.5. Pahlow and Weissbach (1999) have shown that the average FC of unwilted legumes is 27, indicating to their poor ensilability. By the opinion of Pahlow *et al.* (2002), silage material has good ensiling properties if the fermentation coefficient is higher than 45. In our investigation, fermentation of the studied silage material was low and various additives were used to increase it.

The chemical composition of silage is shown in Table 1. The content of dry matter of silages was 140 to 171 g/kg. The data of Hetta (1999) also show that the dry matter of silages made from clover-timothy was low. The content of dry matter in control silage and in that with biological additives was 126 g/kg and 130 to 166 g/kg, respectively.

As compared to the control silage, silages treated with biological or chemical additives had higher content of dry matter, crude protein and nitrogen-free extractives and lower content of crude fibre (P<0.0001). The content of crude protein was significantly higher while using biological additives I-2 and AIV − 173 g/kg and 172 g/kg, respectively (P<0.0001). As microorganisms use organic matter − especially carbohydrates − for their life activity during fermentation, changes occur in the chemical composition of silage (McDonald *et al*., 1991).

Data about silage digestibility, DM losses and fermentation parameters are presented in Tables 2 and 3. DM losses were quite high, reaching to 24.1% in the control silage. The data of Pettersson (1988) also show that DM losses of the silage with low DM content are high – 0.8 to 71.1%, the average 19.4% – depending on the silage material. All additives reduced DM losses during fermentation. DM losses were the lowest (7.7%) with additive I-3, being by 3.1 times lower than that of untreated silage. All starter bacteria improved silage fermentation, resulting in reduced DM losses. Several researchers (Gallo *et al*., 2002; Jatkauskas and Vrotniakiene, 2005) have shown the same results in their studies with silages prepared from red clover or its mixture and treated with a biological additive.

Organic matter digestibility of silage improved significantly with the use of additives (P<0.0001). The digestibility of the control silage in comparison with the treated silages was by 10% lower, probably resulted by silage spoilage (pH 5.9) which decreases digestibility (Muck, Pitt, 1993). Jatkauskas and Vrotniakiene (2005) have revealed that organic matter digestibility of silage from red clover and timothy mixture, treated with a biological additive, is 76%, while the respective figure for untreated silage is 74.8%.

Table 1. The effect of biological and chemical additives on the chemical composition of silage (g/kg DM)

Test silage	Dry matter	Crude protein	Crude ash	Crude fibre	N-free extractives
Control	140	153	109	274	421
I-1	169	167	90	217	476
I-2	163	173	92	213	470
I-3	171	161	86	220	481
I-4	168	162	90	224	473
AIV	162	172	91	222	471
Significant difference, P					
control *vs*. I-1	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
control *vs*. I-2	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
control *vs*. I-3	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
control *vs*. I-4	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
control *vs*. AIV	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
I-1 *vs*. I-2	0.0121	0.0073	0.2087	0.5022	0.1063
I-1 *vs*. I-3	0.4819	0.0008	0.0042	0.1726	0.1322
I-1 *vs*. I-4	0.5541	0.0040	0.5424	0,0238	0.3331
I-1 *vs*. AIV	0.0027	0.0432	0.8188	0,0604	0.1316
I-2 *vs*. I-3	0.0022	<0.0001	0.0002	0.0477	0.0035
I-2 *vs*. I-4	0.0452	<0.0001	0.0682	0,0049	0.4966
I-2 *vs*. AIV	0.5378	0.5288	0.2996	0,0139	0.8454
I-3 *vs*. I-4	0.2012	0.5263	0.0179	0,3236	0.0177
I-3 *vs*. AIV	0.0005	<0.0001	0.0024	0,5771	0.0056
I-4 *vs*. AIV	0.0115	<0.0001	0.4039	0.6621	0.6263

Table 2. The effect of biological and chemical additives on silage pH, ammonia-N, DM concentration and OM digestibility

Test silage	pH	NH$_3$-N total N %	DM losses, %	OMD *in vitro*, %
Control	5.9	18.7	24.1	66.0
I – 1	4.1	1.1	8.6	76.2
I – 2	4.1	2.0	11.7	75.7
I – 3	4.0	1.1	7.7	76.0
I – 4	4.1	1.3	9.0	74.6
AIV 2000	4.5	5.8	11.7	73.4
Significant difference, P				
Control *vs*. I-1	<0.0001	<0.0001	<0.0001	<0.0001
Control *vs*. I-2	<0.0001	<0.0001	<0.0001	<0.0001
Control *vs*. I-3	<0.0001	<0.0001	<0.0001	<0.0001
Control *vs*. I-4	<0.0001	<0.0001	<0.0001	<0.0001
Control *vs*. AIV 2000	<0.0001	<0.0001	<0.0001	<0.0001
I-1 *vs*. I-2	0.4082	0.0027	0.0154	0.4983
I-1 *vs*. I-3	0.0161	0.6863	0.3607	0.8528
I-1 *vs*. I-4	1.0000	0.4934	0.7062	0.0332
I-1 *vs*. AIV	<0.0001	<0.0001	0.0050	0.0006
I-2 *vs*. I-3	0.0022	0.0010	0.0017	0.6216
I-2 *vs*. I-4	0.4082	0.0142	0.0355	0.1290
I-2 *vs*. AIV	<0.0001	<0.0001	0.6328	0.0033
I-3 *vs*. I-4	0.0161	0.2804	0.2015	0.0491
I-3 *vs*. AIV	<0.0001	<0.0001	0.0005	0.0010
I-4 *vs*. AIV	<0.0001	<0.0001	0.0122	0.1047

The indicators of silage fermentation quality – high pH, high content of butyric acid, low content of lactic acid and high level of ammonia-N in total-N – reveal that the ensilability of the untreated silage material was low (Tables 2 and 3). The pH value of well-fermented high-quality silage with DM 200 g/kg should be 4.2 or lower (Weissbach, 2003). Lower pH value of the silage treated with a biological additive, compared to the one treated with chemical additive (P<0.0001), was resulted by a quite high concentration of lactic acid bacteria (8×10^9 cfu/g). The proportion of ammonia-N in TN shows the intensity of protein degradation. The studies by Winters *et al*. (2002) and Rajčákova *et al*. (2005) have shown that proteolysis decreases in silages treated with a

biological additive (*L. plantarum*). Both biological additives and AIV promoted lactic acid fermentation, increasing thus the content of lactic acid ($P<0.0001$ and $P<0.001$) and decreasing the content of alcohol, butyric acid and buthandiol as compared to the control silage (Figure 1). In comparison with the biological additive, the chemical additive significantly decreased the alcohol level in silage ($P<0.0001$). The positive effect of biological additives in ensiling red clover with low DM content has been shown by several authors (Gallo, *et al*., 2002, 2003, 2006; Speijers *et al*., 2002, Rajčákova *et al*., 2005) who claim that a biological additive improves fermentation quality.

Table 3. The effect of biological and chemical additives on the content of ethanol, lactic, acetic and butyric acids (g/kg DM)

Test silage	Ethanol	Lactic acid	Acetic acid	Butyric acid
Control	37	14	8	47
I-1	14	46	9	3
I-2	15	71	12	1
I-3	17	43	7	1
I-4	15	59	8	0
AIV	6	39	9	2
Significant difference, P				
Control *vs*. I-1	<0.0001	<0.0001	0.4098	<0.0001
Control *vs*. I-2	<0.0001	<0.0001	0.0034	<0.0001
Control *vs*. I-3	<0.0001	0.0001	0.1798	<0.0001
Control *vs*. I-4	<0.0001	<0.0001	0.6290	<0.0001
Control *vs*. AIV	<0.0001	0.0003	0.5620	<0.0001
I-1 *vs*. I-2	0.5789	0.1500	0.0238	0.0109
I-1 *vs*. I-3	0.1640	0.9197	0.0361	<0.0001
I-1 *vs*. I-4	0.5713	0.2732	0.1966	<0.0001
I-1 *vs*. AIV	<0.0001	0.6863	0.8041	0.9435
I-2 *vs*. I-3	0.3912	0.1251	0.0001	0.0434
I-2 *vs*. I-4	0.9910	0.7177	0.0010	0.0003
I-2 *vs*. AIV	<0.0001	0.0701	0.0136	0.0129
I-3 *vs*. I-4	0.3973	0.2331	0.3811	0.0411
I-3 *vs*. AIV	<0.0001	0.7616	0.0605	<0.0001
I-4 *vs*. AIV	<0.0001	0.1390	0.2920	<0.0001
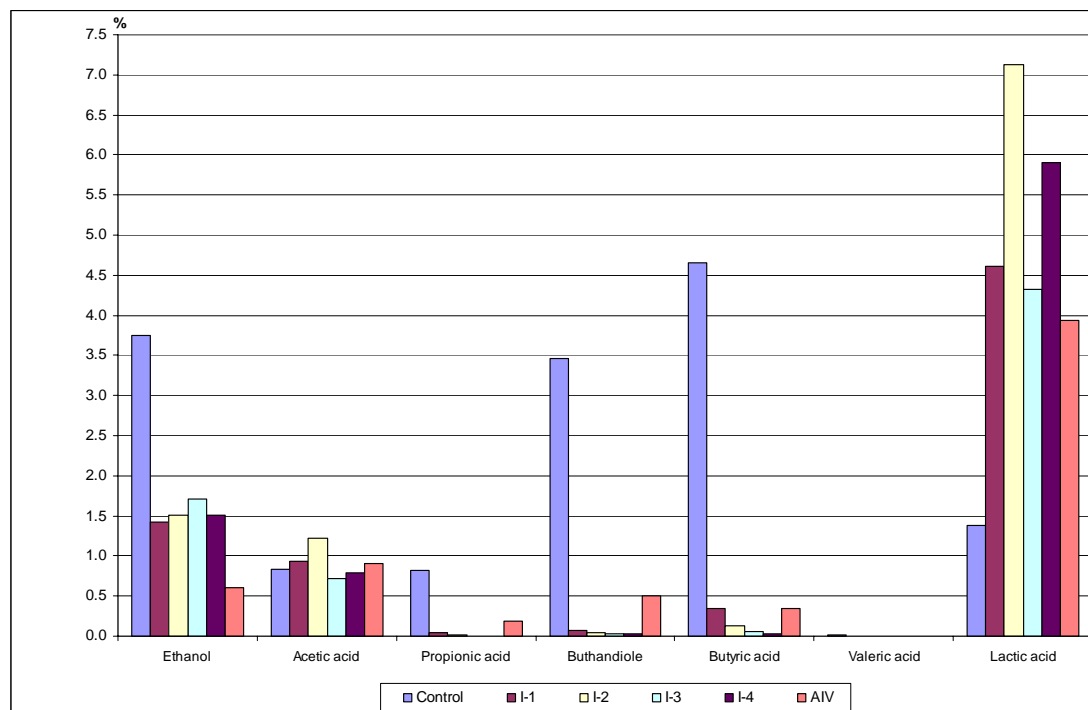
Figure 1. The content of ethanol, buthandiol, lactic and other volatile fatty acids in test silages

Summary

    Due to the low fermentation coefficient (27.5) of red clover and timothy mixture it is necessary to use additives in order to make high-quality silage from it. The use of biological or chemical additives decreased silage DM matter losses by 2.1 to 3.1 times. DM losses were the lowest (7.7%) when additive I-3 was used.

    Treatment with a biological additive with lactic acid bacteria concentration 8×10^9 cfu/g significantly improved the fermentation quality of red clover-timothy silage: pH 4.0–4.1, ammonia-N in TN 1.1–2.0%, lactic acid content 43–71 g/kg and butyric acid content 0–3 g/kg in DM.

    Organic matter digestibility of the treated silage improved up to 10% as compared to that of the untreated silage (P<0.0001).

    All studied biological additives improved the ensilability of clover-timothy mixture with a low DM content. Biological additive I-3 appeared to be the most suitable for treating that kind of silage material.

References

AOAC. 1990. Official methods of analysis. 15th ed. Association of Official Analytical Chemists International, Arlington,VA, p. 68–88.

Doyle, C. J., Topp, F. E. 2002. An economic assessment of the potential for increasing the use of forage legumes in north European livestock systems. – Legumes silages for animal production: LEGSIL. Eds. R. J. Wilkins, C. Paul. Braunschweig, Germany, p. 75–85.

Faithfull, N. T. 2002. Methods in agricultural chemical analysis: a practical handbook. – CABI Publishing, UK, 266 pp.

Gallo, M., Jambor, V., Mlynár, R., Rajčáková, L. 2002. Effect of the application of different silage preparations upon the fermentation process in red clover. – Proceedings of the XIIIth International Silage Conference, Auchincruive, Scotland, p. 110–111.

Gallo, M., Rajčáková, L, Mlynár, R. 2003. The effect of applicatin biological additive on fermentation quality of red clover silage. – Proceedings of the 11th International Scientific Symposium of Forage Conservation, Nitra, Slovak Republic, p. 96–97.

Gallo, M., Rajčáková, L., Mlynár, R. 2006. Effect of application biological additives on fermentation quality of red clover silage. – Proceedings of the 12th International Scientific Symposium of Forage Conservation, Brno, Czech Republic, p. 214–216.

Harman, E., Kleinmans, J., Riser, B., Rutherford, W. 1999. Distribution and activity of microbial inoculants applied at reduced volumes with yhe Pioneer Appli-Pro[TM]Application Systems. – Proceedings XIIth International Silage Conference. Uppsala, Sweden, p. 92–93.

Hetta, M. 1999. Ensiling during difficult conditions of two direct cut forages, with different botanical composition. – Proceedings XIIth International Silage Conference. Uppsala, Sweden, p. 94–95.

Jatkauskas, J., Vrotniakiene, V. 2005. Performance of lactating dairy cows fed rations with inoculated red clover-grass mixture silage. – Integrating efficient grassland farming and biodiversity. Eds. R. Lillak, R. Viiralt, A. Linke, V. Geherman. Greif printhouse, Tartu, p. 494–497.

Jatkauskas, J., Vrotniakiene, V., Miettinen, H. 2002. Comparison of fermentation quality and nutitive value of inoculanted and non-treated legume-grass silages. – Proceedings of the International Conference of Animal Nutrition , Tartu, p. 64–70.

McDonald, P., Henderson, A. R., Heron, S. J. E. 1991. The biochemistry of Silage. Chalcomble publications, Bucks, UK. 340 pp.

Muck, R. E., Pitt, R. E. 1993. Ensiling and its effect on crop quality. – Proceedings of National Silage Production Conference, Syracuse, New York, p. 55–66.

Muck, R. E., Shinners, K. Y. 2001. Conserved forage (silage and hay): progress and priorities. – Proceedings of the XIX International Congress. Sao Pedro, Sao Paulo, Brazil, p. 753–762.

Pahlow, G., Weissbach, F. 1999. New aspects of evaluation and application of silage additives. – Landbauforschung Voelkenrode SH 206, p. 141–158.

Pahlow, G., Rammer, C., Slottner, D., Touri, M. 2002. Ensiling of legumes. – Legumes silages for animal production: LEGSIL. Eds. R. J. Wilkins, C. Paul. Braunschweig, Germany, p. 27–31.

Pettersson, K. 1988. Ensiling of forages. Factors affecting silage fermentation and quality. – Dissertation, Uppsala, 75 pp.

Rajčáková, L., Mlynár, R., Gallo, M. 2005. The influence of the application of a biological additive on the fermentation process of red clover silage. – Silage production and utilization. – Proceedings of the XIVth International Silage Conference, July 2005, Belfast, Wageningen Academic Publishers, p. 203.

Ruser, B., Rutherford, W. M. 1999. Silage quality is affected by lactic acid bacteria strain combinations and dosage. – Proceedings XIIth International Silage Conference. Uppsala, Sweden, p. 113–114.

Speijers, M. H. M., Fraser, M. D., Fychan, R., Theobald, V. J., Winters, A. 2002. Evaluation of different silage additives for ensiling lucerne and red clover. – Proceedings of the XIIIth International Silage Conference, Auchincruive, Scotland, p. 112–113.

Thomas, T. A. 1977. An automated procedure for the determination of soluble carbohydrates in herbage. – J. Sci. Food Agric. 28, p. 639–642.

Van Soest, P. J., Robertson, J. B., Lewis, B. A. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. – J. Dairy Sci., vol. 74, p. 3583–3597.

Weissbach, F. 2003. Theory and practice of ensuring good quality of silages from grass and legumes. – Proceedings of the 11th International Scientific Symposium of Forage Conservation, Nitra, Slovak Republic, p. 31–36.

Winters, A., Lloyd, J., Leemans, D., Lowes, K., Merry, R. 2002. Effect of inoculation with *Lactobacillus plantarum* on protein degradation during ensilage of red clover. – Proceedings of the XIIIth International Silage Conference, Auchincruive, Scotland, p. 108–109.