

# Effect of different dry matter and biological additives application on fermentation process in red clover silages

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## ABSTRACT

Influence of dry matter and application of biological silage additive on the basis of Live system on fermentation process in produced silages was studied in experiment with red clover. It was found out that with increasing amount of dry matter and application of silage additive improved parameters of fermentation process in produced silages. Fermentation process of feed ensilaged with lower dry matter content (27.92 %) was more intensive than of feed with higher content of dry matter (43.32 %). The greatest effect of application of biological additive was observed with ensilaging the feed with content of dry matter 42.32 %. During conservation of silage with higher content of dry matter was observed also the lowest level of proteolysis. Treated silages had lower pH, higher content of lactic acid, lower content of acetic and butyric acid, and % NH3-N of total N compared with non-treated silages.

Keywords: red clover, clover silage, biological additive, live system

## INTRODUCTION

Legumes are a protein source in ruminant nutrition. The capacity of legumes to fix nitrogen from the air results in high protein contents, particularly in lucerne and red clover.

Interest in leguminous forages is increasing as farms reduce their reliance on purchased concentrate feeds and search for improved production from homegrown forages. Red clover is a high yielding, high protein legume than can be grown successfully in submontane and mountainous regions of Slovakia.

For successful ensiling, the ensilability of a crop must be considered. To ensure rapid development of the naturally occurring LAB, adequate levels of readily available substrates in the form of WSC are required. The main sugars present in the WSC fraction of legumes are fructose, glucose and sucrose (McDonald et al., 1991).

Moreover, the buffering capacity, i.e. the plant's ability to resist pH changes, must be sufficiently low, not to counteract the acidity that will be the result of the activity of LAB. The higher the buffering capacity the more acids must be produced by LAB through fermentation to lower

the pH. The concentration of WSC in the aqueous phase will be of importance for the production of lactic acid.

Red clover is high protein forage. Moore and Patterson (2005) reported that red clover silage has a high crude protein content of 16 % to 20 % and a ME content of 10 to 12 MJ/kg DM, depending on the growth stage at cutting.

The quality of clover silage is affected by poor climatic conditions in crop time. Pahlow et al. (2000) states that increase of dry matter ensilaged legumes above 25 % decreased the risk of butyric acid production in non-treated silages.

The aim of this experiment was to study the effect of application a biological and a biological-enzymatic additive on fermentation quality and digestibility of red clover silages with different wilting levels of the forage.

## **MATERIAL AND METHODS**

Two identical covers of tetraploid red clover from the second cut were ensilaged under laboratory conditions. The first cover was ensilaged 24 hours after cut. The second cover was ensilaged 48 hours after cut.

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Following wilting the matter was chopped, homogenized and filled into 1.7 l laboratory silos. The fermentation was observed in untreated control (U), and one experimental variant (T) treated with a biological additive on Live system basis (Lactobacillus plantarum 3676, 3677, and Propionic bacterium DSM 9576, 9577) in both studies. The applied amount was 2.0 ml solution of additive per kilogram of feed.

The silos were placed in standard conditions at 22 oC. Silage losses of dry matter were determined at regular 21-days intervals.

After 180 days of fermentation were the samples of silages determined for nutrient content, dry matter content losses in % of the original dry matter and electrometrically for silage extract pH and in vitro digestibility of dry matter and of organic matter. Lactic acid, acetic acid, propionic

acid, butyric acid, valeric acid and capronic acid levels were determined by gas chromatography, alcohol and NH3 by the microdiffusion method according to Conway. Total volatile fatty acid, total acid was stated out of the determined concentrations.

The results were statistically processed and compared using one-factorial variance analysis and the program Statgraphics.

## **RESULTS AND DISCUSSION**

Different wilted cut red clover had 279 and 423 g dry matter. Next value of clover nutrient composition before conservation shows the table 1. The ensilaged mass had good content of crude protein, higher content of crude fibre and lower content of water-soluble carbohydrate.

Table 1:	Content of	nutrients	in fresh	matter o	f red clover

Level of DM content	Dry matter	Organic matter	Crude protein	Crude fibre	ADF	NDF	Hemi- cellulose	Nitrogen- free extract	Sugar total	Fat	Ash
	g	g . kg <sup>-1</sup> dry mater									
Low	279.21	919.31	195.03	271.92	359.99	431.95	71.96	428.55	58.05	23.82	80.69
High	423.12	914.11	201.95	286.73	377.41	429.29	51.88	403.50	94.09	21.94	85.89

Different dry matter content in ensilaged matter influenced also the course and character of fermentation process (Table 2). In both variants – treated and nontreated – intensive fermentation is characteristic for the stand ensilaged with lower dry matter content. In treated silage we found lower pH, % NH3-N of total N, higher content of lactic acid and lower content of all other acids compared with the non-treated silage. Difference in pH, concentration of lactic and acetic acids, and % NH3 of total N was among the groups statistically highly significant.

More intensive fermentation process of better quality in treated silage became evident also in lower losses of dry matter and higher content of residual sugars in the produced feed. Detected differences are minimal in other parameters of nutrient content.

Increase of dry matter in ensilaged matter became evident by higher pH and totally lower content of acids in treated and non-treated silages as well as by reduction of dry matter losses as opposed to silage with lower content of dry matter. Also smaller amount of acids and lower content of NH3-N of total N detected in silages with higher content of dry matter is related to it. Application of silage additive presented itself positively also in this case. We detected in treated silages lower pH, lower losses of dry matter and lower content of NH3-N of total N compared with non-treated silage. Content of lactic acid was higher in treated silages than in non-treated silages. Content of other acids was lower in treated silage than in the non-treated one. Differences in pH, losses of dry matter, lactic acid and NH3-N out of total N were statistically highly significant among groups.

We did not find essential differences from the viewpoint of nutrient content.

Different amounts of dry matter in ensilaged matter influence markedly the course of fermentation process and its quality as well. It is necessary to choose the way of treatment of ensilaged matter in dependence on the amount of dry matter. Lättemäe and Tamm (2002) and Lättemäe et al. (2006) report that with low contents of dry matter on the level 15 - 18 % it is very effective to treat the ensilaged matter with chemical additive.

Speijers et al. (2002) found a comparable effect between chemical and biological treatment in form of Live system at conservation of red clover with content of dry matter 24.5 %. Other biological additives showed lower quality of fermentation process. These findings correspond with our results as well as with our previous knowledge (Gallo et al. 2002 and 2003).

Another factor influencing markedly the quality of fermentation process is besides dry matter also the content of sugar and age of stand. Lower content of sugar and higher content of fibre in ensilaged stand became evident in our experiment in lower level of fermentation as we detected in our previous work (Gallo 2003). Winters et al. (2002) who studied the influence of biological additive on fermentation of red clover with lower content of sugars give worse results of fermentation process in red clover silage compared with our values. In agreement with our previous work Gallo et al. (2001) we detected better results in conservation of red clover with biological additive than in non-treated silages. As in our previous works also in this one was confirmed positive effect of biological ensilaging additives in conservation of red clover with higher content of sugars even with lower dry matter content.

Application of the biological ensilaging additive on the basis of Live system influenced positively the quality of fermentation process in clover with lower as well as higher content of dry matter. Level of fermentation process is very markedly influenced not only by content of dry matter but also by content of sugars. Feed with lower content of dry matter ferments more intensively than feed with higher content of dry matter.

		Level of DM content								
Parameter			Low				High			
n = 6		U	U		T1		U		T1	
		X	S	X	S	X	S	$\overline{\mathbf{X}}$	S	
DM	in g	262.99	2.47	265.34	2.24	400.30	2.40	407.44	2.82	
СР	in g.kg <sup>-1</sup> DM	192.14	0.94	192.20	2.40	191.90	1.85	191.94	0.89	
CF	in g.kg <sup>-1</sup> DM	288.92	4.86	281.71	4.59	310.22	5.87	303.03	3.53	
ADF	in g.kg <sup>-1</sup> DM	357.65	3.14	353.40	5.98	384.01	4.96	382.31	8.02	
NDF	in g.kg <sup>-1</sup> DM	400.17	4.22	400.70	6.03	423.31	3.44	425.90	6.04	
Sugar total	in g.kg <sup>-1</sup> DM	14.41	3.74	21.05	6.69	27.77	2.48	28.40	2.37	
Fat	in g.kg <sup>-1</sup> DM	29.46	0.91	30.07	2.41	26.99	1.88	26.02	1.15	
Ash	in g.kg <sup>-1</sup> DM	87.35	0.97	85.18	0.41	90.68	0.87	90.20	0.91	
PH		4.45	0.02	**4.14	0.15	4.94	0.11	**4.39	0.05	
DM losses	in %	6.46	0.89	5.25	0.80	6.12	0.55	**4.14	0.64	
Lactic acid	in g.kg <sup>-1</sup> DM	58.12	6.32	**86.75	12.14	27.69	2.80	**48.51	10.32	
Acetic acid Propionic acid	in g.kg <sup>-1</sup> DM in g.kg <sup>-1</sup> DM	13.18 0.14	1.10 0.07	**7.61 0.12	1.69 0.07	8.32 0.07	1.08 0.06	8.65 0.15	1.04 0.10	
Butyric acid Valeric acid Capronic acid	in g.kg <sup>-1</sup> DM in g.kg <sup>-1</sup> DM in g.kg <sup>-1</sup> DM	0.41 0.20 0.11	0.14 0.09 0.04	0.30 0.18 0.15	0.02 0.07 0.08	0.35 0.08 0.05	0.26 0.03 0.02	0.29 0.10 0.02	0.13 0.05 0.01	
NH3-N of total N in %		9.24	0.22	**6.73	0.94	8.24	2.84	**5.77	0.20	
DM digestibility	y in %	60.34	0.60	60.52	0.76	57.15	0.41	57.09	0.36	
OM digestibility	y in %	56.84	0.66	57.11	0.75	53.47	0.37	53.21	0.44	

 $\rm U-untreated,\,T1$  - treated with biological additive,  $\rm CP-crude$  protein.  $\rm CF-crude$  fibre

\* P < 0.05 \*\* P < 0.01 The statistics is performed for the individual trials only.

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