

## The effect of additives on the silage leaches quality of red clover preserved at low dry matter

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*Abstract:* The work deals with the evaluation of the quality of silage made from two red clover varietes (*Trifolium pratense* L.) - diploid varieties (Spurt) and tetraploid varieties (Amos). Experimental stands were established in locations Troubsko and Vatín. There were evaluated silages from the first cuts. The harvest was performed at the beginning of flowering (in late May and early June). The experimental silages were treated with a mixture of organic acids (formic acid and propionic acid). There were evaluated silage liquors (pH, lactic acid, acetic acid, butyric acid, ethanol and NH<sub>3</sub>). The results showed that the content of fermentation acids, pH, ammonium and ethanol was not affected by variety (P <0.05). There was observed the influence of a treatment on the content of acetic acid and ethanol (P <0.05). The used preservative had a positive impact on the final quality of the silage.

*Key-Words:* red clover, silage, low dry matter, organic acids, ethanol

#### Introduction

To make high quality of silage is necessary to use forage of high quality. The better quality of roughage can means reduce of the cost of feeding day. By using of silage additives we can improve quality and there is also a favorable effect on the palatability of the resulting silage. In contrast, silages with lower quality may have a negative impact not only on palatability, but mainly on the health and productivity of animals [2, 11].

The principle is that the higher moisture in the silage means more intensive and spontaneously fermentation. It also causes the production of higher amounts of fermentation products. Fermentation products are a result of microbial activity [14].

The fermentation process is evaluated by the degree of proteolysis, which is great importance in terms of health, performance and reproduction. In our agricultural practice over the last 7 years has become customary to begin harvesting alfalfa and clover in the early formation of flower buds. The result is a silage which has a high content of nitrogen compounds. In this phenological growth has the vegetation low viscosity and wilting is very slow. In this silages with low viscosity can occur the buffering of silage leading to increase of pH above 4.6. This causes a high content of nitrogen

compounds and potassium and at low osmotic pressure is started rapid proteolysis process supported by the activaties of clostridia [12].

Red clover is in agricultural practice, also known as red clover. Red clover is one of the basic fodder rich in proteins and vitamins. This perennial herb with a deep taproot in contrast to alfalfa is characterized by slower intensity of lignification. Despite the relatively short persistence in the stand (2-4 years) is red clover one of the most important forage. Red clover ensures the production of quality forage and improves soil quality [10]. The requires of red clover are soil with surface compaction and sufficient supply to the moisture [5].

It is an ideal crop for green feed. Red clover also thrives on the less fertile soils where alfalfa grows no longer, well tolerates and acidic soils. Red clover can be used in multiple ways - in the mix, for purposes grazing, for hay or silage. When drying there may be a problem with mashing at the higher layers during stock. In the Czech Republic is registered 40 varieties - 19 diploid and 21 tetraploid [10].

Tetreploid varieties characterized in compare with diploid varieties with higher production of fresh forage, increased stamina and increased competition in mixtures with grass species. The



forege of tetraploid varietes aging more slowly, is about 7 to 10 days later, which allows sequential harvesting for direct feeding of fresh forage [6].

Most of our diploid varieties is adapted to the environment, is less demanding and early. Tetraploid varieties have green forage yield about 12-20% higher and hay yield is higher of 2-3%. Crude protein content is higher of 4-5%. They are persistent and have a higher carbohydrate content. Most of them are later, slower aging and contain more water [17].

The aim of this study was to assess the quality of silage leachate of red clover varieties, diploid varieties (Spurt) and tetraploid varieties (Amos) and their suitability for silage.

#### **Material and Methods**

Small trial plots of red clover varieties SPURT (2n) and AMOS (4n) were founded in the Foraging Research Station of Vatín on the Bohemian-Moravian Highlands (Czech Republic), altitude 560 m above sea and at the Research Institute for Fodder in Troubsko by Brno (Czech Republic, altitude 270 m above sea level. Stands were used as three cuts. There were evaluated silages from the first cuts. The harvest was performed at the beginning of flowering (in late May and early June).

The experimental silages were prepared in containers with a diameter of 150 mm. Preparation of experimental micro silages describes in his work [20]. Silage samples were taken 60 days after ensiling.

Evaluated was the quality of the extracts (pH, lactic acid, acetic acid content, the content of the butyric acid, ammonia and ethanol). Analytical procedures including preparation of aqueous extract describing in work of [1]. The results were recalculated to 100% of dry matter. Results were evaluated by analysis of variance (ANOVA)

followed by Tukey's test. The evaluation was carried at a significance level of P < 0.05.

#### **Results and discussion**

The content of lactic acid was in the variety Amos 101.53 g.kg<sup>-1</sup> and in the variety Spurt 111.03 g.kg<sup>-1</sup> DM g.kg<sup>-1</sup> of dry matter. The content of acetic acid was 27.60 g.kg<sup>-1</sup> of DM, respectively 33.40 g.kg<sup>-1</sup> of dry matter (Table. 1). When comparing the impact

dry matter (Table. 1). When comparing the impact of different varieties there were no significant differences between diploid and tetraploid red clover variety. Tetraploid varieties of red clover should produce more water [17]. The average values of dry matter suggest that but difference was not statistically significant. The silage of Amos varieties showed lower pH and lactic acid but these differences were not statistically significant (Table. 1). Butyric acid in the majority of the samples ranged zero values. Butyric acid was only detected in two samples of the untreated variants of diploid red clover (SPURT).

After treatment of silages by chemical preservative there occurred acidification and their pH reduction from 4.38 to 4.12 (Table. 2). According to [19] the pH in the ideal silage should by fall within the values 4 - 4.2. Doležal [3] discloses a pH of 3.7 - 5. The decrease in pH to 4 -4.5 is also suitable measures against listeria in silage [5]. The optimum of pH values also achieved Silage samples that were not treated with a chemical additive. After addition of chemical occurred also decreasing of a lactic acid content. Lactic acid is an indicator of the quality and stability of the silage so is not so desirable decrease of lactic acid. Kotal [9] states that the lactic acid content in the silage should be 2/3 of the total amount of acids. This also indicates Zeman [21] in tables for evaluating the quality of silage, which specifies the minimum content of lactic acid 70% of the total acids.

Table 1 The influence of the type of dry weight, pH, lactic acid, acetic acid and ethanol [g.kg-1]

Varieties	Dry matter	pН	Lactic acid	Acetic acid	Ethanol		
Amos 4n	159.59 <sup>a</sup>	4.19 <sup>a</sup>	101.51 <sup>a</sup>	27.60 <sup>a</sup>	8.39 <sup>a</sup>		
Spurt 2n	173.44 <sup>a</sup>	4.31 <sup>a</sup>	111.03 <sup>a</sup>	33.40 <sup>a</sup>	$10.78^{a}$		

Different letters in the columns indicate statistically significant differences at a level of P < 0.05

Table 2 Effect of treatment on dry weight. pH. lactic acid. acetic acid and ethanol [g.kg-1]

Treatment	Dry matter	pН	Lactic acid	Acetic acid	Ethanol
Control	164.94 <sup>a</sup>	4.12 <sup>a</sup>	85.53 <sup>a</sup>	22.53 <sup>a</sup>	5.43 <sup>a</sup>
Kemisile	168.09ª	4.38 <sup>a</sup>	127.00 <sup>a</sup>	38.47 <sup>b</sup>	13.73 <sup>b</sup>

Different letters in the columns indicate statistically significant differences at a level of P < 0.05

These values were both variants of experimental silages. After addition of organic acids were reduced (P <0.05) the acetic acid in both monitored varieties. Chemically treated silages also contained significantly less (P <0.05) ethanol. The average value of ethanol decreased from 13.73 g.kg<sup>-1</sup> to 5. 43 g.kg<sup>-1</sup> (Tab. 2).

After the addition of organic acids reacted both varieties of clover by reducing the concentration of acetic acid. The interaction between variety and treatment was significant (P<0.05). The optimal acetic acid content of the total content of acids in silages should be 20 to 30% of dry matter [19]. Drevjany [4] indicates the proportion of acetic acid 4-9 g.kg<sup>-1</sup> in the dry state from 35 to 35%. The acetic acid content is dependent on the number of cuts. On the second and each further mowing forms less acetic acid than in the first cuts [16]. Kocián [8] indicates that the acetic acid content should be within 20 g.kg<sup>-1</sup> of DM. At this concentration is desirable in silage because it keeps the aerobic stability. The concentration of acetic acid over 30 g.kg-1 of DM is associated with high energy losses and reduced feed intake. Also Doležal [3] states the acetic acid content of 30 g.kg<sup>-1</sup>. Formation of acetic acid in silages can prevent wilting and less time adding of silage additives [8]. In both variants of silages there were desired values of acetic acid (Tab. 2). More favorable acetic acid content contained silage from Amos varieties.

Fig. 1 The acetic acid content in red clover silages and interaction between variety and treatment



By using of chemical additives were reduced (P<0.05) ethanol content in both varieties. Spurt and Amos. The interaction between variety and treatment was significant (P<0.05). Wilkinson [19] reported the ethanol content <10 g.kg<sup>-1</sup> of dry matter. Mitrik [10] presents the content of ethanol

4.06 g.kg<sup>-1</sup>. The corresponding valuesof ethanol reached only variants treated by organic acids (Tab. 2). Higher content of ethanol may be a manifestation of the presence of yeast which fermented residual sugars. or lactic acid. Optimum ethanol content in silages should be according to Doležal [2] 8-10 g.kg<sup>-1</sup>. Such content has a positive effect on palatability and cows preferred such silage. The high content of ethanol may negatively affect aerobic stability of silage and the rumen microflora [14].

Fig. 2 The ethanol content in red clover silages and interaction between variety and treatment



### Conclusion

The evalueted experimental micro silages of red clover had comparable pH. fermentation acids. ammonia and ethanol. These results were achieved in both studied varieties (diploid varieties Spurt and tetraploid varieties Amos). Based on the results it can be assumed that for a production of silage can be used both diploid and tatraploid varieties of red clover. A silage quality can be influenced by using a chemical preservative. There was a significant effect on the concentration of acetic acid and ethanol content in monitored silages after a chemical preservative. This effect was observed for both varieties of red clover.

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#### References:

- [1] Doležal P, Vliv přídavku Lctobacillus plantarum DSM 12771 na kvalitu siláží silně zavadlé vojtěšky a trávy (Effect of supplements of Lactobacillus plantarum DSM 12771 on the quality of ensiled alfalfa and grass with a high kontent of dry metter). *Acta univ. agric. et silvic Mend.* Bruno. 5. 2002. s. 37 – 44.
- [2] Doležal P, et al.. Konzervace krmiv a jejich využití ve výživě zvířat. Olomouc: Petr Baštan. 2012. 307 s. ISBN 978-80-87091-33-3.
- [3] Doležal P, Zeman L, Dvořáček J, Posuzování hygienické kvality krmiv. Zemědělec: Hygienická a zdravotní rizika krmiv [online]. 2012. č. 43 [cit. 2013-04-17]. Dostupné z: http://www.agroweb.cz/Posuzovanihygienicke-kvality-krmiv s1708x61791.html
- [4] Drevjany L, Kozel V, Padrůněk S, *Holštýnský svět.* ZEA Sedmihorky. 2004. 346 s.
- [5] Dvořáčková J, Význam zdravotní nezávadnosti siláží. Zemědělec: Hygiena krmiv a zdravotní rizika [online]. 2010. č. 7 [cit. 2013-04-6]. Dostupné z: http://www.agroweb.cz/Vyznamzdravotni-nezavadnostisilazi s531x45300.html
- [6] Gerhard V, et al., *Grünlandwirtschaft und Futterbau*. Stuttgart: Ulmer. 1987. ISBN 38-001-3071-8.
- [7] Hrabě F, Buchgraber K, *Pícninářství travní porosty*. MZLU v Brně. Brno. 2009. 154 s.
- [8] Kocián J, Krmte studenou. hygienicky nezávadnou siláž!. Náš chov. 2013. LXXIII. č. 4. 36 - 38.
- [9] Kotal V, Výživa a krmní hospodářských zvířat: Učební text pro střední zemědělské technické školy oboru pěstitelsko - chovatelského. Druhé přepracované vydání. Praha: Státní zemědělské nakladatelství. 1962. 267 s.
- [10] Mitrík T, Ako zvládnuť úskalia silážovania ďatelovín a vyrobiť kvalitnů siláž?. Náš chov. 2013. LXXIII. č. 3. 54 - 56.
- [11] Pelikán J, Hýbl M, Rostliny čeledi Fabaceae LINDL. (bobovité) České republiky: (se zvláštním zaměřením na druhy významné pro zemědělství). Troubsko: Zemědělský výzkum Troubsko. 2012. 230 s. ISBN 978-80-905080-2-6.

- [12] Peymanfar S, Kermanshahi RK, The effect of bacteria. enzymes and inulin on fermentation and aerobic stability of corn silane. *Iran Journal Microbiol.* 4. s. 180–186. 2012
- [13] Pozdíšek J, Metodická příručka pro chovatele k výrobě konzervovaných krmiv (siláží) z víceletých pícnin a trvalých travních porostů: metodika. 1. vyd. Rapotín: Výzkumný ústav pro chov skotu. 2008. 38 s. ISBN 978-80-87144-06-0.
- [14] Rada V, Vlková E, Silážní inokulanty. Praha: Výzkumný ústav živočišné výroby. 2010. 57 l. ISBN 978-80-7403-069-7.
- [15] Rajčáková L, Gallo M. Problémy silážovania d'ateliny lúčnej. *Slovenský chov.* 2005. X. č. 3. s. 24-26.
- [16] Santos EM, Pereira OG, Garcia R, Ferreira CLLF, Oliveira JS, Silva TC, Rosa LO, Microbial populations. fermentative profile and chemical composition of signalgrass silages at different regrowth ages. *Revista Brasileira de Zootecnia*. 2011. 40(4). s. 747-755. ISSN 1806-9290. DOI: 10.1590/S1516-35982011000400007.
- [17] Skládanka J, Multimediální učební texty pícninářství. in: [online]. [cit. 2014-10-07]. Dostupné z: <u>http://web2.mendelu.cz/af\_222\_multitext/picni</u> ny/sklady.php
- [18] Šantrůček J, *Encyklopedie pícninářství*. ČZU v Praze. Praha. 2008. 157 s.
- [19] Wilkinson JM, *Silage*. Lincoln: Chalcombe Publications. 2005. ISBN 09-486-1750-0.
- [20] Vyskočil I, Skládanka J, Doležal P, Havlíček. Z, Metodika výroby experimentálních mikrosiláží. Brno: Mendelova univerzita v Brně. 2011. 23 s. ISBN 978-80-7375-543-0
- [21] Zeman L, et al.. Výživa a krmení hospodářských zvířat. 1. vyd. Praha: Profi Press. c2006. 360 s. ISBN 80-867-2617-7.