CHEMICAL AND MICROBIAL QUALITY OF ALFALFA AND RED CLOVER MIXTURE SILAGES DEPENDING ON THE SHARE IN THE MIXTURE AND ADDITIVES

Lazarević, Đ.[1], Đorđević, N.[2], Stevović, V.[3], Tomić, D.[3], Anđelković, S.[1], Marković, J.[1], Prijović, M.[1]

[1]Institute for Forage Crops Kruševac, Miodraga Petrovića Čkalje 266, Kruševac-Globoder, Serbia
[2]Faculty of Agriculture, University of Belgrade, Nemanjina 6, Belgrade-Zemun, Serbia
[3]Faculty of Agronomy, University of Kragujevac, Cara Dušana 34, Čačak, Serbia

ABSTRACT

This research was conducted to study the influence of ensiled alfalfa and red clover mixtures, mixed at different ratios, as well as the effect of additives – two doses of oak tannin extract and bacterial inoculant on the basic chemical composition and the number of different microorganisms of the silages. With increasing the share of red clover in mixture, there was a significant reduction in proteolysis. Treatments with 50 and 75% red clover had the highest number of lactic acid bacteria and the lowest number of total bacteria. When tannins and LAB inoculants were added, a significant reduction in the level of proteolysis was also observed, which compared to the control treatment was up to 25%.

Keywords: additive, alfalfa, red clover, silage

INTRODUCTION

When alfalfa is ensiled alone, the fermentation outcomes are often suboptimal, because of its high buffering capacity and low sugar and dry matter concentrations (Wang *et al.*, 2019). During the production of alfalfa silage occurs the process of proteolysis in significantly higher extent in comparison with red clover silage (Beauchemin *et al.*, 1997). Jones *et al.* (1995) reported reduction of proteolysis by 70% when mixing extracts of red clover and alfalfa at 1:1 ratio, compared with untreated alfalfa extract in laboratory scale. Many researches connect this fact with presence of polyphenol oxidase in red clover. The positive effect of alfalfa ensiling in a mixture with red clover could also be achieved through richness in vitamins, minerals and other substances, as these two plant species complement each other in terms of these substances (Chiriac *et al.*, 2020). Treatments with commercially available silage additives, such as tannins, could effectively reduce non-protein nitrogen (NPN) formation during ensiling (Tabacco *et al.*, 2006). According to Pys *et al.* (2002), a significant inhibition of protein proteolysis was found in alfalfa-rolled barley silage made with the addition of lactic acid bacteria (LAB) inoculants. This paper discusses the effects of different additives and species share in mixture on the chemical composition and microbial status of alfalfa and red clover mixture silages.

MATERIALS AND METHODS

Green forage was cut in 2024 at the beginning of flowering. The experiment, involving treatments as combinations of six different mixtures and four additives, was performed. The following mixtures were included: alfalfa (A1), alfalfa and red clover at a ratio of 90:10 (A2), 70:30 (A3), 50:50 (A4), 30:70 (A5), and red clover (A6) on a fresh (wilted) matter (FM) basis. The additive factor variants were as follows: control (without additive) (B1), two doses of granulated oak tannin extract (6 g/kg DM (B2) and 12 g/kg DM (B3); 'Essedielle SRL', Italia), and bacterial inoculant 'BioStabil Plus' which contained homofermentative lactic acid bacteria (*Enterococcus faecium* and *Bacillus plantarum*), and heterofermentative lactic acid bacteria (*Bacillus brevis*) with a concentration of 5×10^{10} CFU per gram (B4). The mixtures were ensiled in the polyethylene jars holding 5 dm^3, with three replications. After 56 days the experimental silos were opened and representative samples were taken for chemical analyses.

In the silage following chemical parameters were determined: crude protein (CP), crude fiber (CF), ether extract (EE), crude ash (CA), nitrogen free extract (NFE) and water soluble nitrogen (H_2O-N). NDF and ADF were determined applying the method of Van Soest *et al.* (1991). The following microbial parameters were analyzed: total bacteria count (TB), lactic acid bacteria count (LAB), and yeast and mold count (YM). Statistical analysis of the obtained results was done by analysis of variance procedure with software package STATISTICA, version 8.0 ('STATISTICA 8', Stat. Soft. 2007).

RESULTS AND DISCUSSION

With an increase in the proportion of red clover in the mixture, there was a decrease in the crude protein content and significant reduction of proteolysis under the influence of polyphenol oxidase (Table 1). Also, the addition of tannin and inoculant led to a significant reduction of proteolysis, which was consistent with Tabacco *et al.* (2006). Similar to our investigation, Li *et al.* (2018) stated that the crude protein content in silage differed significantly between the mixtures of alfalfa and red clover, and ranged from 163.1 g/kg DM in pure red clover silage to 183.7 g/kg DM in pure alfalfa. According to Contreras-Govea *et al.* (2013), the ADF content of the control variant of alfalfa silage was 227.7 g/kg DM, while the ADF content of the variant with the inoculant *Lactobacillus plantarum* was 218.5 g/kg DM silage, which supports the results of our experiment. Other variations of the examined chemical parameters can be explained by the relative change in their mutual relationship, due to the loss of volatile ingredients (ammonia, free acetic and butyric acid) when preparing the samples by drying in accordance with AOAC methods, as well as due to the extraction of part of the lactic acid with diethyl ether when determining the fat content (Đorđević *et al.*, 2011).

CONCLUSION

From the results it is evident that addition of red clover to alfalfa when ensiling, as well as the use of oak tannins and inoculants led to a significant reduction of proteolysis, which is considered the most important effect of the tested factors on the quality and nutritional value of silage. The determined trend is particularly significant due to the fact that alfalfa is the most important and cheapest source of protein in rations for dairy cows in Serbia, and the obtained values should be used when balancing the ration in accordance with current normatives. The variation of other parameters of the chemical composition was mostly insignificant, and of no importance for the use of silage. A more detailed examination of the influence of the type and dose of tannins is planned, due to the possibility that, in excessive amounts, they may have a negative effect on protein utilization.

https://doi.org/10.11118/978-80-7701-093-1-0114

Tab. 1: Chemical composition of silages (g/kg DM) and number of microorganisms (log cfu/g)

	CP	CF	EE	NFE	CA	NDF	ADF	H2O-N	LAB	TB	YM
Mixture											
A1	195.8^{ab}	295.4	40.6	377.2	90.8	506.6	400.7	387.4^{a}	8.02^{b}	8.66^{a}	2.82^{b}
A2	196.7^{ab}	298.8	37.9	374.9	91.6	505.5	403.1	365.1^{b}	8.10^{ab}	8.62^{ab}	2.61^{c}
A3	199.6^{a}	298.1	38.7	373.3	90.4	508.9	402.3	380.0^{ab}	7.99^{b}	8.60^{bc}	2.64^{bc}
A4	197.2^{ab}	295.2	38.2	378.1	91.3	506.4	403.2	311.3^{c}	8.16^{a}	8.54^{d}	2.81^{b}
A5	197.2^{ab}	295.3	39.0	376.1	92.4	505.7	399.8	315.7^{c}	8.17^{a}	8.57^{cd}	2.80^{bc}
A6	187.2^{b}	296.8	39.5	387.1	89.3	503.3	400.3	300.0^{c}	8.10^{ab}	8.59^{bcd}	3.02^{a}
Additive											
B1	193.4	297.7^{a}	38.0	377.4	93.3	507.2	405.3^{a}	405.3^{a}	8.11	8.60	2.68^{b}
B2	194.4	297.0^{ab}	38.1	380.9	89.5	505.6	402.8^{ab}	337.7^{b}	8.02	8.58	2.83^{ab}
B3	196.5	300.6^{a}	39.4	373.3	90.1	507.1	400.6^{ab}	327.1^{b}	8.11	8.62	2.84^{a}
B4	198.1	291.1^{b}	40.4	379.6	90.8	504.5	397.6^{b}	302.7^{c}	8.12	8.59	2.79^{ab}
ANOVA											
A	*	ns	ns	ns	ns	ns	ns	**	*	**	**
B	ns	*	ns	ns	ns	ns	*	**	ns	ns	*
AXB	ns	ns	ns	ns	ns	ns	ns	**	**	**	**

Values in columns with a different uppercase letters are significantly different based on the LSD test (P<0.05); F-test: * – P<0.05; ** – P<0.01; ns – F-test not significant.

Acknowledgement

This research was financed by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia, project ref. number 451-03-33/2026-03/200217.

REFERENCES

Beauchemin, K. A., Rode, L. M., Eliason, M. V. 1997. Chewing activities and milk production of dairy cows fed alfalfa as hay, silage, or dried cubes of hay or silage. *Journal of Dairy Science*. 80, 324–333.

Chiriac, E. R., Chițescu, C. L, Sandru, C., Geană, E. I., Lupoae, M., Dobre, M., Borda, D., Gird, C. E., Boscencu, R. 2020. Comparative Study of the Bioactive Properties and Elemental Composition of Red Clover (Trifolium pratense) and Alfalfa (Medicago sativa) Sprouts during Germination. *Applied Sciences*. 10, 7249.

Contreras-Govea, F. E., Muck, R. E., Broderick, G. A., Weimer, P. J. 2013. Lactobacillus plantarum effects on silage fermentation and in vitro microbial yield. *Animal feed science and technology*. 179, 61–68.

Đorđević, N., Grubić, G., Dinić, B., Stojanović, B., Božičković, A. 2011. The Influence of compression level and inoculation on biochemical changes in lucerne silages. *Journal of Agricultural Sciences*. 56(1), 15-23.

Jones, B. A., Muck, R. E., Hatfield, R. D. 1995. Red clover extracts inhibit legume proteolysis. *Journal of the Science of Food and Agriculture*. 67, 329–333.

Li, X., Tian, J., Zhang, Q., Jiang, Y., Wu, Z., Yu, Z. 2018. Effects of mixing red clover with alfalfa at different ratios on dynamics of proteolysis and protease activities during ensiling. *Journal of Dairy Science*. 101, 1–11.

Pys, J., Migdal, W., Pucek, T., Zivkovic, B., Fabjan, M., Kosovac, O., Radovic, C. 2002. Effect of lactic acid bacterial inoculant with enzyme and rolled barley additive on the chemical compostion and protein degradation of alfalfa silage. *Biotechnology in Animal Husbandry*. 18, 33–44.

StatSoft Inc. 2007. S*TATISTICA (data analysis software system), version 8.0*. www.statsoft.com.

Tabacco, E., Borreani, G., Crovetto, G.M., Galassi, G., Colombo, D., Cavallarin, L., 2006. Effect of chestnut tannin on fermentation quality, proteolysis, and protein rumen degradability of alfalfa silage. *Journal of Dairy Science*. 89, 4736–4746.

Van Soest, P. J., Robertson, J. B., Lewis, B. A. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*. 74, 3583-3593.

Wang, M., Wang, L., Yu, Z., 2019. Fermentation dynamics and bacterial diversity of mixed lucerne and sweet corn stalk silage ensiled at six ratios. *Grass and Forage Science*. 74(2), 264–73.

Contact Information

Đorđe Lazarević: email: djordje.lazarevic@ikbks.com