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THE EFFECT OF CARBOHYDRATE ADDITIVE AND INOCULATION ON QUALITY OF RED CLOVER SILAGE

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Abstract: In this experiment, wilted masses of red clover of cultivar K-17 from the first cut was ensiled in three treatments: a) no additives, b) with the addition of corn (6% of biomass) and c) with the addition of inoculant BioStabil Plus. The experiment design was according to the method of a completely random plan (single factorial trial) in triplicates. Based on the results it can be concluded that the wilted biomass of red clover can be successfully ensiled without additives. However, the inoculation of red clover biomass achieves the most favourable pH value (4.20), the lowest level of degradation of the protein expressed in the amount of NH_3-N (107.7 gkg^{-1} N), the largest production of lactic acid (91.3 gkg^{-1} DM) and acetic acid (42.6 gkg^{-1} DM), in the absence of butyric acid. Adding maize meal in the amount of 6% contributed to somewhat more favourable fermentation and increase of the energy value of silage. When using the DLG and Weissbach methods for assessing the quality of silage, all silages were classified into the first class. Contrary to this, according to the Zelter method, control and inoculated silages were evaluated as class III, because of the large amounts of acetic acid. In practices inoculants based on homo-and hetero-fermentative bacteria of lactic acid fermentation are recommended for use, because the increased production of acetic acid contributes positively to the aerobic stability of silage.

Key words: silage, red clover, corn meal, inoculant, quality

Introduction

The use of conserved forages (hay and silage) throughout the year, combined with the required amount of concentrate, is generally accepted and widespread trend in countries with developed cattle breeding. The fact that the quality hay

depends on weather conditions caused the hay to be the feed of the most variable chemical composition and nutritional value (*Antov et al., 1994*). Methods of mechanical conditioning of biomass accelerate storage (drying) of hay (*Savoie et al., 1997*), and the use of chemical preservatives (propionic acid-based) enables storing of the mass with higher moisture content (*Bolsen, 1993*). Nevertheless, the most effective way to preserve maximum nutritional value of plant mass of the first cut is ensiling. In this regard, in Serbia there are numerous experiences with alfalfa, while the ability of red clover ensiling was far less studied. Although it is superior forage crop to alfalfa due to its remarkable biological properties, red clover is a significant potential for food production in areas with unfavourable pH (*Dinić et al., 2012*).

Conservation of biomass of red clover by method of ensiling, based on lactic-acid fermentation, is difficult because of the low content of fermentable carbohydrates, high buffer value and high moisture content (*Beyer et al., 1982*). These problems are solved, similar to other legumes, by wilting, using carbohydrate supplements/additives, and by using the bacterial inoculants (*Đorđević and Dinić, 2003*). However, the knowledge and experience gained in experiments on alfalfa, can not be used directly in ensiling of red clover, which is specific in regard to certain characteristics of chemical composition (*Đorđević et al., 2012*).

The practice of ensiling requires maximum simple, cheap and effective procedures. Accordingly this experiment is planned with the aim to investigate the effect of the use of carbohydrate supplement (corn meal) and bacterial inoculant on chemical composition and quality parameters of red clover silage.

Material and Methods

In the experiment, the biomass of wilted red clover of the first cut, cultivar K-17 at the start of flowering phase was ensiled. The average dry matter content at ensiling time was 331.7 g kg-1. The experiment was set up as a random plan (single factorial trial) with three replications: a) control (RC_C - Red Clover control), b) the addition of 6% of corn (RC_{+MM} - Red Clover + maize meal) c) addition of inoculant Biostabil Plus (RC_{+I} - Red Clover + inoculant). Plus BioStabil is the inoculant of the Austrian company Biomin containing homo-fermentative lactic acid bacteria (*Enterococcus faecium, and Bacillus plantarum*) and hetero-fermentative lactic acid bacteria (*Bacillus brevis*) at a concentration of 5×10^{10}cfu per gram. Ensiling was carried out in experiment containers holding 130 dm^3.

Chemical analyses of samples of initial material, maize meal and silage were conducted in the laboratory of the Institute of forage crops in Kruševac, according to standard methods (*AOAC, 2002*). Parameters of biomass suitability for ensiling (mono saccharides, soluble carbohydrates and buffer capacity) were determined by the method of *Weissbach (1967)*. Minimum content of DM which guarantees

obtaining of stable silage without the presence of butyric acid was calculated according to the formula Y (g/kg) = 450 – (80 × S/BC) by *Beyer et al. (1982).*

In the biomass of red clover, maize meal and silage the following were analytically determined: dry matter (DM), crude protein (CP), crude fibre (CF), crude fat (CL), NDF, ADF, ash, Ca and P, and nitrogen-free extracts (NFE) were calculated. The silage DM content was determined, the degree of acidity (pH), ammonia nitrogen (NH_3-N), the content of acetic, butyric and lactic acid. In the assessment of silage quality three methods were used: DLG, Zelter and Weissbach (*Đorđevic and Dinić, 2003*). Nutritional value expressed in units of NE_L and NE_M was calculated according to *Obračević (1990),* and digestibility coefficients of nutrients according to *Glamočić (2002).* On the basis of chemical composition (CP, ADF, NDF) and dry matter digestibility, the relative feed value (RFV) was obtained according to the standards of quality for legumes and grass - American Forage and Grassland Council (*Schroeder, 1994*). The results of chemical analysis were analyzed by variance analysis, and statistical significance of differences was tested by LSD test (*StatSoft, 2006*).

Results and Discussion

Suitability of biomass for ensiling

Suitability of plants for silage can be decided on the basis of the ratio of sugar quantity and buffer capacity (*Dinić et al., 1998*). If the sugars present were used for the synthesis of lactic acid with 100% efficiency, the ratio of sugar quantity to the buffer capacity (S/BC) could be 1.0. However, the natural micro-flora can transform only about 50 % of the sugar into lactic acid, which means that the S/BC ratio must be greater than 1.0. This relationship depends on the level of dry matter in the ensiled material. In particular S/BC ratio, production of lactic acid is even greater if the dry matter content is higher (*Beyer et al.,1982*).

Suitability of biomass for silage is being tested in order to forecast the quality of silage prepared without additives. Determined content of soluble carbohydrates (sugars), substances essential for lactic acid fermentation, in the present experiment was 132 gkg^{-1}DM and buffer capacity (BC) was 59.7, while the ratio S/BC 2.23. *Weissbach (1967)* has stated that the above ratio must be greater than 3.0 in order to obtain stable silage without the presence of butyric acid. Contrary to this, the results from this experiment showed that stable and high-quality silage can be obtained under significantly narrower S/BC ratio. S/BC ratio of 2.23 requires a minimum dry matter content of 282 gkg^{-1}, while in the experiment DM of 332 gkg^{-1} was determined, i.e., was significantly higher.

Table 1. Suitability of red clover biomass for ensiling, gkg^{-1} DM

Type of material	DM, gkg^{-1}	Sugars, gkg^{-1} DM	Buffer capacity, meq/100 g DM	S/BC	Required DM, gkg^{-1}
Red clover	332	133	59,7	2,23	282
Maize meal	875	167	30,5	5,48	-

Sugar content in the biomass of red clover in this experiment was slightly higher than the results of previous research (106-124 gkg^{-1} DM), obtained by *Dinić et al. (1994),* while the values for BC were almost identical (58-60 meq/100 g DM lactic acid).

Content of nutrients in the initial material and silage

The chemical composition of the biomass of red clover and maize meal is shown in Table 2. At the time of ensiling, biomass of red clover contained 168.3 gkg^{-1}DM of crude protein and less than 250 gkg^{-1} DM crude fibre which provided good nutritional value and good digestibility. Maize meal contained higher concentration of CF (41.8 gkg^{-1} DM). Compared to the initial material, the control silage and silage with maize meal contained lower amount of CP, while the treatment with inoculants had almost the same amount of CP. Crude fat content was higher in silages compared to the initial material, which could be explained by forming of the lower fatty acids in the fermentation process (Table 2). NDF and ADF in the initial material were similar to that of the control silage, slightly lower than in silages with additives. There was no statistically significant difference in the content of NDF and ADF in silage.

Based on the content of ADF of 338.6 gkg^{-1} DM, and according to quality standards for legumes and grasses, green mass of the material was evaluated I quality class, and according to the NDF content of 482.5 gkg^{-1} DM for the same criterion it was ranked in the second quality class.

The calculated nutritional value of green mass expressed in units of NEL and NEM (Table 2) was significantly higher than the nutritional value of silage. This difference was a result of higher values of the digestibility coefficient of the green mass compared to silage.

Relative feed value (RFV) of the initial material was 120 points, 117 for the control silage and silage with maize meal 124 points, and accordingly classified as Class II quality, whereas silage with inoculants was evaluated with 130 points and evaluated as the first class quality.

Table 2. Chemical composition and nutritional value of initial material and red clover silage, gkg^{-1} DM

Sample	CP	CF	CL	NFE	NDF	ADF	NEL MJkg^{-1}	NEM MJkg^{-1}	RFV
Initial material									
Red clover	168.3	242.9	31.6	443.5	482.5	338.6	5.54	5.56	120
Maize meal	100.1	41.8	46.2	769.8	-	-			
Silage									
RC_C	156.9 ^{b}	267.6 ^{a}	40.7 ^{a}	430.0 ^{b}	491.8 ^{a}	348.2 ^{a}	5.17	5.04	117
RC_{+MM}	161.5 ^{b}	236.6 ^{b}	45.0 ^{a}	452.5 ^{a}	475.9 ^{a}	325.9 ^{a}	5.23	5.13	124
RC_{+I}	168.7 ^{a}	249.1 ^{b}	41.8 ^{a}	435.7 ^{b}	462.5 ^{a}	313.7 ^{a}	5.19	5.07	130
LSD 0,05	7.07	18.15	8.57	16.64	30.01	36.49	-	-	-
LSD 0,01	10,07	27,43	12,99	25,21	45,48	55,30	-	-	-

Differences in the contents of CL, ADF, NDF, mineral substances, Ca and P between the different treatments were not statistically significant (Tables 2 and 3). Statistical significance was found in regard to the CP content between the silage with inoculants on the one hand and silage without additives and with the addition of maize meal on the other (Table 2). Inoculant provided better preservation and less degradation of CP compared to other treatments. Control silage had significantly highest content of CF.

The amount of CP determined in this trial was lower than the previously determined value by *Dinić et al. (1994),* which amounted to 180 and 192.5 gkg^{-1} DM. The amount of CF in the experiment (about 250 gkg^{-1} DM) was higher compared to the results of *Svirskis (2005),* which were in the range 16.69-21.25% and *Đukić et al. (2007),* which amounted to 195.6 gkg^{-1} DM. Slightly lower content of CP in DM of the first cut red clover (149.1-163.1 gkg^{-1}) were determined by *Dinić (1990),* also *Vasiljević et al. (2012)* (14.9-16.5%), and significantly higher in DM of the second cut obtained by the same authors (16.1 to 18.7%). The nutritive value of the initial material and silage (contents of CP, CF, NFE, NDF, ADF, RFV, etc.) largely depend on the stage of cutting of plants, as confirmed by the research results of *Ignjatović et al. (2003), Marković et al. (2010)* and *Vasiljević et al. (2012).*

Maize meal contained a small amount of total mineral matters and calcium in relation to red clover. The Ca: P ratio in red clover DM was very unfavourable and amounted to 6:1. *Dinić et al. (1994)* have found a more favourable ratio of Ca and P (about 4.0-4.5: 1) in the initial material and in silage.

Table 3. Content of mineral substances in the initial material and red clover silages, gkg^{-1} DM

Samples	Ash	Ca	P
Initial material			
Red clover	113.7	18.1	3.0
Maize meal	19.3	2.1	3.0
Silage			
RC_C	104.8 [a]	20.6 [a]	3.3 [a]
RC_{+MM}	104.3 [a]	19.1 [a]	3.4 [a]
RC_{+I}	104.9 [a]	20.5 [a]	3.0 [a]
LSD 0,05	10.68	4.45	0.77
LSD 0,01	16.19	6.74	1.18

Biochemical changes in the silage

The amount of dry matter in all silages was higher than 300 gkg^{-1}. Differences in DM were significant between treatments, and the highest amount of DM was established for the treatment RC_{+MM}, which was contributed by the addition of maize meal (Table 4).

There was a statistically significant difference in the degree of acidity between the control silage and silages with additives, with the most favourable pH value (4.20) determined in silage with inoculants (Table 4). The amount of NH_3-N expressed in terms of total nitrogen is an indicator of protein degradation in the process of ensiling, and high significance for this parameter between the control silage and silages with additives was determined. Silage with inoculants had the lowest value for ammonium nitrogen, which can be explained by a low degree of proteolysis in the middle with the lowest pH value. The level of dry matter and pH are the most important factors that determine the intensity of proteolysis, but they cannot completely stop it (*Carpintero et al., 1979*).

Table 4. Parameters of biochemical changes in red clover silages

Treatments	DM, gkg^{-1}	pH	NH_3-N, gkg^{-1} N	Acid content in silages					
				Acetic		Butyric		Lactic	
				gkg^{-1} DM	% TA	gkg^{-1} DM	% TA	gkg^{-1} DM	% TA
RC_c	303.3c	4.67a	167.6a	34.2ab	36.31	1.7a	1.80	58.3b	61.89
RC_{+MM}	346.6a	4.37b	138.2ab	23.9b	25.34	0.4a	0.42	70.0b	74.23
RC_{+I}	323.3b	4.20b	107.7b	42.6a	31.81	0.0a	0.00	91.3a	68.19
LSD 0,05	13.98	0.172	38.3	13.98	-	1.92	-	18.83	-
LSD 0,01	21.20	0.260	58.0	21.20	-	2.92	-	28.53	-

Legend: TA – Total acids

Relative amount of lactic acid in all silages was greater than 60% (relative to the total acidity), which allows for the maximum number of points (20) according to the DGL method for evaluating of the quality. The amount of butyric acid was highest in the control silage (1.8%) and it was scored 9 points (out of a maximum 10) by DLG method. The highest absolute values for the amount of lactic acid and acetic acid (91.3 and 42.6 gkg^{-1} DM) were found in silage with inoculants (Table 4), which is a result of homo-and hetero-fermentative lactic acid bacteria from used inoculants. Increased content of acetic acid negatively affects the quality class of silage. In this experiment, it is certainly a result of the use of inoculants with hetero-fermentative bacteria of lactic acid fermentation and it is significant in a positive way due to increased aerobic stability of silage (*Hu et al., 2009*).

The results of these studies, in regard to the degree of acidity and acetic acid content were in the range of results obtained by *Dinić et al. (1994)* (pH 4.29 and the acetic Acid 8.66-9.98 gkg^{-1}). Less favourable values in this study were for the % of $\%NH_3$-N/ΣN (5.78-9.07%), and favourable results were found for the content of lactic (14.9 gkg^{-1}) and butyric acid (0.497-0.867 gkg^{-1}) with the dry matter content of 226.3-258.5 gkg^{-1}. Application of inoculant contributed to greater production of lactic and acetic acids and decreased production of butyric acid, which is consistent with research of *Đorđević et al. (2004)*.

In a more realistic assessment of the quality of silage three methods were used: DLG, Zelter and Weissbach (Table 5). According to the method of Weissbach and DLG, all silages were rated first class. When using the method according to Zelter, silages were evaluated as III class (treatments RC_C and RC_{+I}), and class II (treatment RC_{+MM}), as a result of higher content of acetic acid.

Table 5. Evaluation of the quality of silage using different methods

Treatments	DLG		Zelter		Weissbach	
	Number of points	Class	Number of points	Class	Number of points	Class
RCc	44	I	13	III	90	I
RC_{+MM}	48	I	15	II	95	I
RC_{+I}	49	I	13	III	95	I

Conclusion

Based on the established results in the experiment consisting of ensiling of wilted mass of red clover without additives and with the addition of maize meal (6%) and inoculant, the following was determined:

- Red clover wilted biomass can be successfully ensiled without additives and high quality silage can be achieved (Class I by DLG and Weissbach methods);
- Addition of maize meal in the amount of 6% contributes to somewhat more favourable fermentation and increase of the energy value of silage;

- The use of inoculants containing hetero-fermentative bacteria of lactic acid fermentation has negative impact on the quality of silage (Class III by Zelter method) but it is important for the aerobic stability of silage;

- Inoculation of silage provided the most favourable pH value (4.20), the lowest level of degradation of the protein expressed in the amount of NH_3-N (107.7 gkg^{-1} N), the highest production of lactic acid (91.3 gkg^{-1} DM) and acetic acid (42.6 gkg^{-1} DM), in the absence of butyric acid.

The general conclusion is that wilted biomass of red clover should be ensiled with the addition of appropriate inoculants.

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Uticaj ugljenohidratnog dodatka i inokulacije na kvalitet silaže crvene deteline

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Rezime

U eksperimentu je silirana provenula masa crvene dateline sorte K-17 iz prvog otkosa u tri tretmana: a) bez aditiva, b) sa dodatkom kukuruzne prekrupe (6% od biomase) i c) sa dodatkom inokulanta BioStabil Plus. Eksperiment je postavljen po metodi potpuno slučajnog plana (monofaktorijalnog ogleda) u tri ponavljanja.

Na osnovu utvrđenih rezultata može se zaključiti da se provenula biomasa crvene dateline može uspešno silirati bez aditiva. Međutim, pri inokulaciji biomase crvene deteline postiže se najpovoljnija pH vrednost (4.20), najmanji stepen degradacije proteina izražen kroz količinu NH_3-N (107.7 gkg^{-1} N), najveća produkcija mlečne kiseline (91.3 gkg^{-1} DM) i sirćetne kiseline (42,6 gkg^{-1} DM), uz istovremeno odsustvo buterne kiseline. Dodavanje kukuruzne prekrupe u količini od 6% doprinosi nešto povoljnijoj fermentaciji i povećanju energetske vrednosti silaže. Pri korišćenju DLG i Weissbach metode za ocenu kvaliteta sve silaže su svrstane u I klasu. Nasuprot tome, pri korišćenju Zelter metode, kontrolna i inokulisana silaža su ocenjene III klasom, zbog velike količine sirćetne kiseline.

Za praksu se preporučuje upotreba inokulanata na bazi homo- i heterofermentativnih bakterija mlečnokisleinskog vrenja, jer povećana produkcija sirćetne kiseline pozitivno doprinosi aerobnoj stabilnosti silaža.

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