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THE INFLUENCE OF COMPRESSION LEVEL AND INOCULATION ON BIOCHEMICAL CHANGES IN LUCERNE SILAGES

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Abstract: The effect of different levels of compression (A_1 = 420 gdm^{-1}, A_2 = 560 gdm^{-1}) and inoculation (B_1 = no inoculant, B_2 = with inoculant) on changes in chemical composition, proteolysis and quality of lucerne silage was investigated in this paper. Based on the results of chemical analysis we found that in silages with more compressed material there was a reduction in the amount of ammonia nitrogen, soluble nitrogen and acetic acid, and increased content of protein nitrogen ('true'protein) and production of lactic acid ($p<0.05$). With the inoculation of the ensiling material the production of ammonia nitrogen and acetic acid was reduced but the content of lactic acid and acidity was increased ($p<0.05$). The interaction of both investigated factors (A×B) induced a decrease in the proteolysis degree, increase of lactic acid production and decrease in acetic acid production, and decrease in pH values ($p<0.001$) in investigated silages. The investigated factors had less influence on the chemical composition of lucerne material, and the significant variations were observed in fat and NFE contents. On the basis of this investigation the degree of compression is the most important parameter in ensiling technology. With the adequate compression and reduction of air in the starting material, the aerobic phase is reduced and the activity of proeolytic enzymes is decreased. In practice the special attention should be given to factors on which directly or indirectly the level of compression of ensiled material depends: wilting, cutting, object selection and/or selection of machines used for compression.

Key words: lucerne, compression, inoculation, proteolysis, quality.

Introduction

High moisture content in the moment of cutting and high buffer value are the main problems for a successful ensiling of legumes, and this was the reason for several experiments performed during the past couple of decades (Đorđević and Dinić, 2003). Today, more attention is also given to the changes of nitrogenous

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compounds which occur during the ensiling process (Đorđević et al., 2004). In living plants 75 to 90% of total nitrogen is in the form of true protein, while in silages it is just 30 to 50%, according to Slottner and Bertilsson (2006). Compared to other plants from the *Fabaceae* family lucerne has more soluble nitrogen matters. Protein solubility is in positive correlation with ruminal degradability (Wattiaux, 1995), which may reduce their utilization (Jovanović et al., 1993), or lead to health problems in animals (Koljajić et al., 1997). According to Broderick (1995) other legumes have less soluble proteins compared to lucerne, due to higher presence of condensed tannins. Such results were experimentally obtained by Albrecht and Muck (1991) for sainfoin, lespedeza, and *Lotus pedunculatus*. According to the same authors, species such as red clover or kura clover do not contain condensed tannins, but their proteins have low solubility. The lesser degree of proteolysis in red clover silages was explained by the presence of soluble enzyme polyphenol-oxidase, which in the presence of oxygen reacts with O-diphenol creating very reactive O-quinone, which creates polymers with other molecules such as proteins (Getachew et al., 2009; Grabber, 2009; Lee et al., 2009).

Various methods are used to maximally control the process of nitrogen matter degradation during the legume ensiling process, such as wilting, carbohydrate stimulation, inoculation and chemical conserving (Nadeau et al., 2000; Guo et al., 2008). Aside from these technologies the cultivars of legumes are selected to have lower degradability (Broderick et al., 2004), and also genetically manipulations were done with the same purpose (Getachew et al., 2009). The ensiling practice requires maximally simple, inexpensive and effective processes. This experiment was planned to investigate the influence of various degrees of compression and inoculation of wilted lucerne material on chemical composition, changes in nitrogen matters and silage quality parameters.

Materials and Methods

The experiment was organized as two-factorial (2×2), with three replications, where factor A was the different level of compression (A_1 = 420 gdm^{-1}, A_2 = 560 gdm^{-1}) and factor B was inoculation (B_1 = no inoculant, B_2 = with inoculant). Lucerne was cut at the end of butonization phase, and the wilted material from the second cut with 330 gkg^{-1} dry matter was used for ensiling.

The inoculation was done with homofermentative inoculants, which according to the producer's specification contained microencapsulated bacteria *Lactobacillus plantarum* (min. 1.0×10^{11} CFU), *Lactobacillus acidophilus* (min. 1.0×10^{11} CFU), *Streptococcus faecium* (min. 1.0×10^{11} CFU) and *Pediococcus acidilactici* (min. 1.0×10^{11} CFU). The quantity used according to the producer's recommendation was 250 g per 5 T of green mass.

All silages after the treatment were compressed in plastic experimental silos with volume of 5 dm^3. After 56 days the experimental silos were opened and representative samples were taken for chemical analyses. A chemical composition and silage quality were analyzed in the Laboratory for nutrition of domestic and reared animals at the Faculty of Agriculture, Zemun (AOAC, 2002). The calculation of evaporated substances in silage dry matter (volatile fatty acids, alcohol, and ammonia) was corrected according to Dulphy and Demarquilly (1981). The amount of ammonia nitrogen was analyzed with modified Kjeldahl procedure (Dulphy and Demarquilly, 1981), amount of soluble nitrogen was analyzed with Vistahin method (Dulphy and Demarquilly, 1981), and protein nitrogen with Grando method (Sinovec and Ševković, 1995). A statistical analysis of the obtained results was done by analysis of variance procedure with software package Statistica v.6. (Statsoft, 2006).

Results and Discussion

The amount of dry matter in all silages was greater than 300 $gkg,^{-1}$ which is considered as the main condition for preventing the separation of juices and maximal control of butyric fermentation (Table 1). Similarly, the increase of dry matter content is important in legumes to preserve proteins (Slottner and Bertilsson, 2006). The chemical composition of silages was not much different from the starting material, just the fat content showed some variation. Almost double quantity of ether extract in silages compared to the starting material can be explained with extraction of the part of lactic acid (which is not volatile) with diethyl-ether (Barnett, 1954). That is the reason why silages from more compressed material had higher fat content. Diethyl-ether also extracts other fat-like substances such as plant pigments, waxes, ether oils, fat soluble vitamins and others (Đorđević et al., 2003), and all this is increasing the calculated fat content. The differences in protein amount between the starting material and silages occurred because of the drying process of the samples during which some ammonia was lost. Compression factor had a significant influence on NFE amounts. In more compressed silages the fermentation was more dominant of the lactic acid type and more fermentable carbohydrates were used to produce lactic acid. In addition, inoculated silages had significantly less NFE, because of more intensive lactic acid fermentation. Nonsignificant variations in crude fiber and ash contents can be treated as relative, because of the variations in other components.

The use of inoculants in this experiment had a purpose of providing homofermentative lactic acid bacteria at the beginning of fermentation, which is scarce. Commercial products used as inoculants are used with the doses that provide 10^5-10^6 lactic acid bacteria per gram of ensiled mass, which enables them to become dominant over the enterobacteria (Prikryl, 1997).

Table 1. Chemical composition of starting material and silages, gkg^{-1} DM.

Compression (A)	Inoculation (B)	Dry matter	Proteins	Fat	Cellulose	NFE	Ash
Starting material		243.46	221.32	44.27	215.73	408.44	110.24
			Silage				
420 gkg^{-1} (A_1)	No inoculant (B_1)	333.08^{ns}	209.16^{ns}	70.43^{b}	206.87^{ns}	403.56^{a}	109.99^{ns}
	With inoculant (B_2)	334.96^{ns}	208.65^{ns}	77.85^{b}	205.83^{ns}	395.96^{a}	111.70^{ns}
560 gkg^{-1} (A_2)	No inoculant (B_1)	337.30^{ns}	209.05^{ns}	83.43^{ab}	205.05^{ns}	392.28^{ab}	110.19^{ns}
	With inoculant (B_2)	331.07^{ns}	212.92^{ns}	91.97^{a}	205.11^{ns}	377.03^{b}	112.82^{ns}
Average for A_1		334.02	208.90	74.14	206.35	399.76	110.84
Average for A_2		334.18	210.98	87.70	205.08	384.66	111.50
Average for B_1		335.19	209.10	76.93	205.96	397.92	110.09
Average for B_2		333.02	210.78	84.91	205.47	386.50	112.26
Average for experiment		334.10	209.94	80.92	205.71	392.21	111.18
Significance for p	Factor A	0.9556	0.2021	0.0041	0.5544	0.0128	0.4959
	Factor B	0.4604	0.3095	0.1448	0.8206	0.0812	0.0083
	Interaction A×B	0.4885	0.1867	0.0054	0.9364	0.0051	0.0578

^{a,b,}A×B-Values in the same column with different letters are statistically significantly different ($p<0.05$); ^{ns} = not significant.

Ammonia nitrogen is one of the most important parameters that reflect changes in nutrient contents. It is produced by the action of proeolytic enzymes from plant cells and microorganisms, mostly butyric *Clostridia*. A presence of ammonia in silages that do not contain butyric acid is a result of plant enzyme activity (McDonald et al., 1991). In all silages made from better compressed material and those inoculated, there was a decrease in ammonia content (Table 2). That is the result of lower pH values, as well as more intensive homofermetative lactic acid bacteria, and more lactic acid produced. The possibility for control ammonia nitrogen production is very significant for lucerne silages, considering that this plant species is very important, sometimes the most important protein source in cow rations (Grubić et al., 2001). Values of pH and dry matter content are the most important factors that dictate the intensity of proteolysis, but they cannot stop it completely (Carpintero et al., 1979). Maximal control of proeolytic processes is achieved with chemical conservants based on organic acids and their

salts (Komprda et al., 1996). However, chemical conservants are used very rarely in Europe and they are banned in the USA (Đorđević and Dinić, 2003). Results of this experiment have a similar trend like previous experiments by Hristov and Sandev (1998), Nadeau et al. (2000), Guo et al. (2008) and Grabber (2009).

In silages with higher degree of compression, significantly less soluble nitrogen was detected and significantly more protein nitrogen. At the same time, there was apparently no influence of inoculation on both parameters. All silages had more than 600 gkg^{-1} of soluble nitrogen, which is considered as the upper limit for quality silages (ENSILAGE, 1978). The influence of degree of proteolysis can be explained with more intensive oxidation in the environment with more oxygen in the material. The result of that is temperature rise, which is in positive correlation with the enzymatic activity. The level of dry matter has smaller significance, but has a direct influence through the compressing ability of plant material (Muck and Diskerson, 1987).

Table 2. Content of ammonia, soluble and protein nitrogen in total, gkg^{-1} N.

Compression (A)	Inoculation (B)	NH_3N	Soluble N	Protein N
420 gkg^{-1} (A_1)	No inoculant (B_1)	220.86[a]	762.71[a]	262.56[c]
	With inoculant (B_2)	197.71[b]	723.41[b]	293.76[b]
560 gkg^{-1} (A_2)	No inoculant (B_1)	197.52[bc]	707.04[c]	307.88[b]
	With inoculant (B_2)	189.34[c]	686.06[d]	324.26[a]
Average for A_1		209.28	743.06	278.16
Average for A_2		193.43	696.55	316.07
Average for B_1		209.19	734.88	285.22
Average for B_2		193.52	704.74	309.01
Average for experiment		201.36	719.80	297.12
Significance for p	Factor A	0.0198	0.0010	0.0011
	Factor B	0.0219	0.0737	0.0868
	Interaction A×B	0.0000	0.0000	0.0000

[a,b,c,d]A×B-Values in the same column with different letters are statistically significantly different ($p<0.001$).

All lucerne silages had high pH values, which is a result of high dry matter content and high buffer value of this plant species, as well as a high content of crude protein and minerals (Table 3). Although those substances have alkaline character, the significant increase in lactic acid and decrease in acetic acid were observed with more compressed material, when inoculants were used, and in the interaction of those factors. Similarly, silages made with more compressed

material had significantly more lactic acid and less acetic acid which can be explained by the shorter duration of aerobic phase of fermentation and better conditions for lactic acid bacteria activity. It was discovered that butyric acid was not present in most silages. That can be explained with the adequate conditions

Table 3. Parameters of biochemical changes in silages, gkg^{-1} DM.

Compression (A)	Inoculation (B)	pH	Lactic acid	Acetic acid	Butyric acid
420 gkg^{-1} (A_1)	No inoculant (B_1)	5.55^{a}	47.32^{c}	66.05^{a}	3.42^{ns}
	With inoculant (B_2)	5.15^{c}	$50.81b^{c}$	57.64^{b}	0.09^{ns}
560 gkg^{-1} (A_2)	No inoculant (B_1)	5.31^{b}	52.30^{b}	57.84^{b}	0.79^{ns}
	With inoculant (B_2)	4.99^{d}	59.93^{a}	53.76^{b}	0.00^{ns}
Average for A_1		5.35	49.06	61.84	1.76
Average for A_2		5.15	56.12	55.80	0.40
Average for B_1		5.43	49.81	61.94	2.10
Average for B_2		5.07	55.37	55.70	0.04
Average for experiment		5.25	52.59	58.82	1.07
Significance for p	Factor A	0.1232	0.0063	0.0313	0.3005
	Factor B	0.0002	0.0476	0.0248	0.1037
	Interaction A×B	0.0000	0.0001	0.0013	0.1774

[a,b,c,d,]A×B-Values in the same column with different letters are statistically significantly different ($p<0.001$).

during the ensiling, and the fact that raw material was not contaminated with soil, which is the main source of butyric *Clostridia* (Đorđević et al., 2004). The explanation lies also in the higher dry matter content in all starting materials, which limits the activity of butyric bacteria.

Conclusion

In the performed investigations, it is found that the degree of compression of ensiling material is more important for the status of nitrogenous matters (ammonia, soluble and protein nitrogen), and for the production of lactic acid than inoculation process. The anaerobity of the environment and optimal conditions for lactic acid bacteria activity is the first and unavoidable condition in ensiling technology. However, the results of this experiment indicate that interaction of this factor with inoculation shows positive results on all parameters of proteolysis and silage quality (aside from butyric acid) with high significance ($p<0.001$). In practice the

maximal attention should be given to factors which directly or indirectly influence compression degree: wilting, cutting length, selection of ensiling object and selection of machines used for compressing (tractor or baling and wrapping).

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UTICAJ STEPENA SABIJANJA I INOKULACIJE NA BIOHEMIJSKE PROMENE U SILAŽAMA LUCERKE

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R e z i m e

U radu je ispitivan uticaj različitog stepena sabijanja (A_1 = 420 gdm^{-1}, A_2 = 560 gdm^{-1}) i inokulacije (B_1 = bez inokulanta, B_2 = sa inokulantom) na promene parametara hemijskog sastava, proteolize i kvaliteta silaže lucerke. Na osnovu rezultata hemijskih analiza utvrđeno je u silažama od bolje sabijenog materijala smanjenje količine amonijačnog i rastvorljivog azota, kao i sirćetne kiseline, i povećanje količine proteinskog azota („pravog“ proteina) i mlečne kiseline ($p<0,05$). Inokulacijom siliranog materijala smanjena je produkcija amonijačnog azota i sirćetne kiseline i istovremeno povećana produkcija mlečne kiseline i kiselost silaža ($p<0,05$). Interakcijom ispitivanih faktora ($A\times B$) došlo je do smanjenja stepena proteolize u silažama, povećanja produkcije mlečne kiseline i smanjenja količine sirćetne kiseline, a time i smanjenja pH vrednosti ($p<0,001$). Ispitivani faktori su bili od manjeg značaja za promene parametara hemijskog sastava hraniva pa su signifikantno varirali samo količina masti i BEM-a. Na osnovu izvedenih ispitivanja može se zaključiti da je stepen sabijanja najvažnija mera u tehnologiji siliranja hraniva. Adekvatnim sabijanjem skraćuje se trajanje aerobne faze i ograničava delatnost proteilitičkih enzima. U praksi treba posvetiti maksimalnu pažnju faktorima od kojih direktno ili indirektno zavisi stepen sabijenosti siliranog materijala: stepenu provenulosti, dužini seckanja, izboru tipa objekta za siliranje i/ili izboru mehanizacije za sabijanje (gaženje ili baliranje).

Ključne reči: lucerka, sabijanje, inokulacija, proteoliza, kvalitet.

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