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INFLUENCE OF MIN-A-ZEL PLUS ON BIOCHEMICAL, MICROBIOLOGICAL AND MYCOTOXICOLOGICAL PARAMETERS OF LUCERNE SILAGE

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Abstract: Lucerne of the 5th cut in the budding phase was ensiled in the experiment as fresh and wilted (DM= 220 and 360 g/kg), with two degrees of compression (520 and 380 g/dm^3) and with added Min-a-zel plus as 2 g/kg green mass. The experiment was performed as the statistical model 2^K.

Min-a-zel plus used as 2 g/kg green mass had no influence on biochemical, microbiological and mycotoxicological parameters of lucerne silage. Compared to the beginning material, silages had more diverse microflora and more mycotoxins. All silages had high pH values and fermentation of the acetic acid type. Some improvement was achieved with wilting of the ensiling material. The least quality (V class) was obtained with fresh lucerne, which was less compressed, while others were ranked as IV quality class.

The obtained results fortify the need for further investigations in developing correct doses of Min-a-zel plus to be used in ensiling of lucerne and other materials.

Key words: lucerne, Min-a-zel plus, microflora, mycotoxins.

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Introduction

Because of its high buffering capacity and low level of fermentable sugars lucerne cannot be ensiled alone. In many experiments the number of solutions are developed to solve that problem: wilting, combining with plant material that is easily ensiled, stimulating of the lactic acid bacteria fermentation with the addition of carbohydrate feeds and bacterial-enzymatic inoculants, chemical conservation. In recent times great attention is given to natural zeolites. These mineral additives have capacity to adsorb mycotoxins and also excess moisture, ammonia and radionuclides. Some results of experiments show that zeolites may have positive influence on the quality of silages made from maize, beet pulp and lucerne. It is assumed that with the binding of excess water zeolites enhance the activity of lactic acid bacteria (Adamović et al., 2001; Koljajić et al., 2002, 2003, Grubić et al., 2003).

The structure of natural zeolites is such that they are good adsorbents of little organic molecules. At the ITNMS Institute, Belgrade, the modification of zeolite was done with the cationic exchange of the surface inorganic cation with organic cation. This exchange changes the surface electricity and hydrophobic properties. The mycotoxin adsorption efficiency of this modified mineral adsorbent (commercial name Min-a-zel Plus) is more than 90% for aflatoxines, zearalenon, ochratoxin A and group of ergot alkaloids.

Material and Methods

The aim of the work was to investigate the influence of Min-a-zel Plus, organically modified mineral adsorbent of mycotoxins, on the presence of microorganisms and results of mycotoxicological investigations in lucerne silages prepared in different conditions (different dry matter content and compression degree). It was assumed that addition of Min-a-zel Plus has influence not only on mould activity and amounts of mycotoxins but also on the intensity of fermentation in the ensiled material.

Lucerne of the cultivar NS – Mediana ZMS from fifth cut in the budding period was used. The experiment was done as 2^K model and the investigated factors were:

A) dry matter content (A_1= fresh lucerne, A_2= wilted lucerne);
B) compression degree ($_{B1}$= maximum compression, B_2= compression decreased by 25%);

Min-a-zel Plus was used as 2 g/kg green mass which was 8 g/kg dry matter.

Experimental plan is shown in Table 1, with details about treatments. The used zeolite was organically modified mineral adsorbent of mycotoxins Min-a-zel Plus produced in ITNMS, Belgrade.

Ensiling was done in autumn conditions within six days. Wilting was done under the roof without the influence of rain. Ensiling was done in experimental

silos of the finely cut material, covered with PVC foil and hermetically sealed. The silos were opened after 60 days and samples for analyses were taken. Proximate analysis was done at the Faculty of Agriculture, Zemun, while microbiological and mycotoxicological analyses were done in Bio-ecological center in Zrenjanin. The silage quality was evaluated by DLG method, statistical analyses was done as 2^K model (Stanković et al., 1990).

T a b. 1. - Experimental plan

A_1 - Fresh lucerne DM = 220 g/kg		A_2 – Wilted lucerne DM = 360 g/kg	
B_1 - Compression 520 g/dm^3	B_2 - Compression 380 g/dm^3	B_1 - Compression 520 g/dm^3	B_2 - Compression 380 g/dm^3

Results and Discussion

On the basis of the results obtained with the proximate analysis (Table 2), it can be concluded that addition of Min-a-zel Plus increased the ash content, which changed the relative proportion of other components. However, differences in the content of certain nutrients can be explained by other reasons too. For example, silages had less NFE compared to original material, which can be explained by the use of fermentable sugars on the part of bacteria for their energy needs. In those processes the short-chain organic acids were produced (lactic, acetic, butyric). Higher proportion of crude lipids in silages than in starting material can be explained by the extraction of part of the non-volatile lactic acid with diethyl – ether (Barnett, 1954). The differences in the content of crude protein and crude fiber was of relative character. The investigated factors had significant influence only on dry matter and ash content in silages.

T a b. 2. - Chemical composition of starting material and silages, g/kg DM

Parameters	Starting material	Silages				F_{exp}
		A_1		A_2		
		B_1	B_2	B_1	B_2	
Corrected DM. g/kg	220.81	214.35	199.55	335.63	335.22	$F_A = 316.80$ $F_B = 1.11$
Crude protein	227.34	208.24	202.05	215.25	221.30	$F_A = 4.60$ $F_B = 0.00$
Crude lipids	43.76	86.05	109.13	104.81	105.41	$F_A = 0.45$ $F_B = 1.11$
Crude fiber	215.39	209.34	212.33	208.27	205.55	$F_A = 1.89$ $F_B = 0.00$
NFE	390.09	352.00	334.83	332.54	331.44	$F_A = 2.02$ $F_B = 1.29$
Ash	123.42	144.37	141.66	139.13	136.30	$F_A = 7022$ $F_B = 1918.25$

$F_{1;1;\ 0.10} = 39.9$; $F_{1;1;\ 0.05} = 161$; $F_{1;1;\ 0.01} = 4052$

The high water content (> 800 g/kg) and crude protein (≅ 220 g/kg DM) in the starting material contributed to the production of small amounts of lactic acid (< 45 g/kg DM) and high pH values (≅ 6). Wilting was not successful in the improving of the mentioned parameters, because it has influence on the reduction of total fermentation and increasing of pH value (Djordjević and Dinić, 2003). In silages made from wilted lucerne the lower content of acetic and lactic acid was discovered. That is the result of the lower resistance of acetic and butyric bacteria on the increase of osmotic pressure (Table 3).

T a b. 3. - Biochemical changes in silages, g/kg DM

Parameters	A_1		A_2		F_{exp}
	B_1	B_2	B_1	B_2	
pH	5.92	5.98	5.81	5.90	$F_A = 1.23$ $F_B = 0.77$
Lactic acid	44.24	32.23	43.19	48.12	$F_A = 0.77$ $F_B = 0.17$
Acetic acid					
Free	16.28	16.39	10.07	8.13	$F_A = 34.75$ $F_B = 0.56$
Bonded	48.82	84.12	38.97	45.20	$F_A = 2.61$ $F_B = 2.04$
Total	65.10	100.51	49.04	53.33	$F_A = 4.13$ $F_B = 1.63$
Butyric acid					
Free	0.00	0.00	0.00	0.00	- -
Bonded	11.19	0.00	3.90	3.90	$F_A = 0.02$ $F_B = 0.26$
Total	11.19	0.00	3.90	3.90	$F_A = 0.02$ $F_B = 0.26$
NH_3-N. g/kg N	216.24	229.69	217.23	212.30	$F_A = 15.03$ $F_B = 109.12$
Soluble N. g/kg N	766.53	808.92	784.60	819.54	$F_A = 0.80$ $F_B = 0.21$

$F_{1;1;\ 0.10} = 39.9$; $F_{1;1;\ 0.05} = 161$; $F_{1;1;\ 0.01} = 4052$

Amounts of ammonia and soluble nitrogen in investigated silages were high and according to Dulphy and Demarquilly (1981) characteristic of the lowest class of silage quality (more than 200 g/kg ammonia N and more than 750 g/kg soluble N). Lower compression, higher moisture content and higher pH values were favorable conditions for protein degradability and ammonia production. The pH value and dry matter content are main factors that influence proteolysis but they cannot stop it completely (Carpintero et al., 1979). As a result of the proteolytic enzyme activity of plant and bacterial cells in less compressed silages there was more soluble nitrogen.

Acetic type of fermentation is characteristic of all lucerne silages. Especially favorable for acetic acid production are high moisture and lower compression (treatment A_1B_2). In contrast, in silages from wilted material the intensity of acetic acid bacteria activity was decreased, which was due to their lower resistance to the increase in osmotic pressure (Table 4). However, for the total domination of lactic acid bacteria, even higher content of dry matter was needed (> 350 g/kg), or the stimulation of lactic acid fermentation with carbohydrate additives (Koljajić et al., 1997).

On the basis of pH value and relative ratio of lactic, acetic and butyric acid, silages were ranked by DLG method. The lowest rank, V class of quality, was given to the silage made from fresh material with lower compression (A_1B_2). Other silages were ranked as IV quality class.

T a b. 4. - Relative ratio of acids (%) and silage quality

Parameters	A_1		A_2	
	B_1	B_2	B_1	B_2
Lactic acid	36.71	24.28	44.93	45.68
Acetic acid	54.01	75.72	51.01	50.62
Butyric acid	9.28	0.00	4.06	3.70
Points	20	15	25	27
Quality class by DLG method	IV	V	IV	IV

As mentioned, wilting lasted relatively long (6 days) because of heavy rains during the ensiling period but also because it was intended to investigate ensiling under adverse weather conditions. The aim was also to enhance the additional contamination of the ensiling material with various aerial microorganisms. In spite of that, the wilted lucerne had less microorganism species compared to fresh lucerne (Table 5). Silages had more diverse microorganism population than the starting material.

Addition of Min-a-zel plus at dose of 2 g/kg fresh mass had not stopped mould activity and production of mycotoxins (Table 6). Silages made from fresh lucerne had more zearalenone compared to those made from wilted material. T-2 toxin was detected only in silages made from fresh lucerne. In the experiment of Adamović et al. (2001) with maize ensiled with 0.2% Min-a-zel Plus there were less mycotoxins in silages where zeolite was added and more lactic acid produced. On the basis of these results, similar dose of Min-a-zel Plus was used but obviously without effect. However, in the investigation of Grubić et al. (2003) the positive effects of the use of natural zeolite were obtained with much higher zeolite doses. This entails that lucerne needs higher doses of Min-a-zel Plus. The establishing of optimal doses of Min-a-zel Plus in lucerne ensiling should be the objective of further investigations. Also, the evaluation of the

feasibility of its use along with additives that enhance lactic acid type of fermentation in lucerne (maize meal, beet pulp, molasses etc.)

T a b. 5. - Presence of microorganisms in the starting material and in silage

	Starting material, 22% DM	Starting material, 36% DM	Silages			
			A_1		A_2	
			B_1	B_2	B_1	B_2
Fungus						
Acremoniella altra			+	+		+
Alternaria alternata	+					
Botryotrichum piluluferum			+	+	+	+
Chaethomium globosum	+		+	+	+	+
Cladosporium herbarum			+			
Epicoccum purpurascens	+					
Fusarium culmorum		+				
Fusarium sporotrichioides	+					
Fusarium sp.	+	+				
Geotrychum candidum				+		
Mucor racemosus			+	+	+	+
Penicillium sp.			+			
Trichocladium opacum				+		
Streptomiceta						
Strepromyces sp.			+	+	+	+
Bacterias						
Bacillus sp. 1				+	+	

T a b. 6. - Mycotoxin content in lucerne silages, mg/kg DM

Mycotoxins	Starting material 22% DM	Starting material 36% DM	Silages				F_{exp}
			A_1		A_2		
			B_1	B_2	B_1	B_2	
Aflatoxine							
Ochratoxine							
DAS		0.69					
T-2			5.06	10.92			$F_A = 7.44$
							$F_B = 1.00$
Zearalenone	1.18	3.58	3.92	6.99	5.81	0.85	$F_A = 0.28$
							$F_B = 0.05$

$F_{1;1;\ 0.10} = 39.9$; $F_{1;1;\ 0.05} = 161$; $F_{1;1;\ 0.01} = 4052$

Conclusion

Min-a-zel Plus used in dose of 2 g/kg green mass had no influence on biochemical, microbiological and mycotoxicological parameters of lucerne silage. All silages had high pH value and fermentation of the acetic acid type. Some improvement was obtained with wilting of the ensiling material. The lowest quality silage was obtained with fresh lucerne that was less compressed.

The obtained results indicate that further investigations are necessary to establish optimal doses of Min-a-zel Plus, including its combination with carbohydrate additives that are usually used for lucerne ensiling.

REFERENCES

1. Adamović, M., Nešić, S, Stoićević, Lj., Tomašević-Čanović, M. (2001): Uticaj organski modifikovanog mineralnog adsorbenta mikotoksina "Minazel Plus" na kvalitet silaže biljke kukuruza. Arhiv za poljoprivredne nauke, 62, 220: 317-324.
2. Barnett, A.J.G. (1954): Silage fermentation. Butter worths publications ltd. 88. Kingsway, London, w.c.2.
3. Carpintero, C.M., Holding, A.J., McDonald, P. (1969): Fermentation studies on lucerne. J.Sci. Food. Agr. 20: 677-681.
4. Dulphy, J. P., Demarquilly, C. (1981): Problemes particuliers aux ensilages. Prevision de la valeur nutritive des aliments des Ruminants, I.N.R.A. 81-104.
5. Djordjević, N., Dinić, B. (2003): Siliranje leguminoza. Institut za istraživanja u poljoprivredi. Beograd.
6. Grubić, G., Djordjević, N., Adamović, M., Koljajić, V. (2003): The influence of zeolite addition on lucerne silage quality. Symposium of Livestock Production with International Participation. Ohrid, June, 2003. Republic of Macedonia. Book of Apstracts, 31.
7. Koljajić, V., Djordjević, N., Grubić, G., Jovanović, R., Pavličević, A., Jokić, Ž., Dinić, B. (1997): Kvalitet silaža spremljenih od leguminoza u zavisnosti od korišćenih postupaka i dodataka. Simpozijum „Naučna dostignuća u stočarstvu '97''. Subotica, 21.-25. april. Zbornik radova, str. 203-219.
8. Koljajić, V., Djordjević, N., Grubić, G., Adamović, M. (2002): Kvalitet silaža sirovih repinih rezanaca sa dodatkom zeolita. Mlekarstvo, 11: 325-330.
9. Koljajić, V., Djordjević, N., Grubić, G., Adamović, M. (2003): The influence of zeolite on the quality of fresh beet pulp silage. Journal of Agricultural Sciences 48, 1: 77-84.
10. Stanković, J., Lakić, N., Ljubanović-Ralević, I. (1990): Zbirka zadataka iz eksperimentalne statistike. Poljoprivredni fakultet, Beograd-Zemun.

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UTICAJ MIN-A-ZELA PLUS NA BIOHEMIJSKE, MIKROBIOLOŠKE I MIKOTOKSIKOLOŠKE PARAMETRE SILAŽA LUCERKE

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R e z i m e

U eksperimentu je silirana lucerka V otkosa u fazi butonizacije kao sveža i provenula (SM = 220 i 360 g/kg), sa dva stepena sabijenosti (520 i 380 g/dm^3) i sa dodatkom Min-a-zela Plus, 2 g/kg zelene mase. Ogled je postavljen po statističkom modelu 2^K.

Min-a-zel Plus korišćen u dozi od 2 g/kg zelene mase nije uticao na biohemijske, mikrobiološke i mikotoksikološke parametre silaža lucerke. U odnosu na početni materijal, silaže su sadržale raznovrsniju mikrofloru i veću količinu mikotoksina. Sve silaže su se odlikovale visokim pH vrednostima i fermentacijom sirćetnokiselinskog tipa. Izvesno poboljšanje je postignuto provenjavanjem biljnog materijala. Najlošiji kvalitet (V klasa) imale su silaže od sveže lucerke sa manjim stepenom sabijenosti, dok su ostale ocenjene IV klasom kvaliteta .

Utvrdjeni rezultati ukazuju na neophodnost nastavljanja ispitivanja u cilju utvrdjivanja optimalne doze Min-a-zela Plus za siliranje lucerke i drugih sirovina.

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