Journal of Agricultural Sciences
Vol. 50, No 2, 2005
Pages 123-129

UDC: 633.31:546.291
Original scientific paper

EFFECTS OF THE USE OF FORMIC ACID IN DIFFERENT DOSES AS THE CONSERVANT IN LUCERNE ENSILING

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Abstract: The influence of formic acid added in three doses (3, 5 and 7 g/kg fresh mass) in the intensity of proteolysis and fermentation in lucerne silages was investigated. On the basis of chemical analyses, it is observed that with the increase of conservant dose the lower pH value, limited proteolysis and increased preservation of proteins was achieved. The amino acid content varied very much due to their degradation and biosynthesis. In treated silages a statistically significant increase of lactic acid was observed while silages with maximum dose of conservant had significantly less free and total acetic acid. Presence of bonded butyric acid only in the control silage had no statistical significance. According to Dulphy and Demarquilly (1981) method, control silage with minimum dose of conservant was estimated as III class and silages with medium and high dose of formic acid were estimated as II quality class.

Key words: lucerne, silage, formic acid, proteolysis, fermentation.

I n t r o d u c t i o n

Lucerne iz one of the most important forage crops in many countries. It provides stable dry matter yield, about 15000 kg/ha and approximately 3000 kg/ha of crude protein after the first year of growth (Mejakić et al., 1997). Besides high crude protein proportion, lucerne herbage is rich in carotene, xanthophile and calcium and represents high-grade forage for all kinds of domestic animals diets. However, high proportion of these nutritious substances is lost due to inappropriate conservation processes.

High buffering value, low amount of sugars and significant content of moisture inhibit production of quality lucerne silage. This is why some special

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The investigation was part of the project TR-6813B financed by Ministry of Science and Enviroment Protection.

procedures or additives, such as wilting, mixing with carbohydrate feeds and biological preparations, combining with plant material easy to ensile or chemical conservation are needed (Koljajić et al., 1997).

Investigation of possibilities of lucerne chemical conservation is more than 100 years old. In many experiments various conservant – mineral and organic acids; their salts and gaseous substances were used more or less success fully. Some of them were patented under various commercial names and are used around the world.

During the ensiling process the quantitative and qualitative changes in a nutrient occur, where for ensiling of legumes the most important are changes in nitrogen compounds, the proteolysis (Djordjević et al. 1996). This is very important in ruminant nutrition because free amino acids and other products of protein hydrolysis are degraded very rapidly in the rumen and part of the ammonia produced may stay unused. Also, high quantity of nonprotein nitrogen in the diet may have negative influence on production, health and animal fertility (Grubić et al. 1996). It is important to have this in mind when calculating and formulating diets. With the use of conservants based on strong mineral or organic acids the inhibition of proteolytic activity is achieved very rapidly and efficiently in the silage (Djordjević et al. 1999).

As a herbivore and ruminant, a roe deer (C*apreolus capreolus L.*) eats a large number of feeds, like pasture or browse, and feed selection depends mostly on the season. Being a selective grazer, it obtains relatively high level of protein and low level of fiber in its diets. Additional feeding is required if high gain is wanted and better quality of trophy, which depends on the quality and quantity of available natural feeds and the season. Additional feeds can be hay, roots and tubers, silage, sugar beet pulp and brewers grains, and of concentrates cereal and legume grains including the feed mixes produced by feed mills (Djordjević et al, 2005). Maize and lucerne silages are very inportant foods for roe deer that likes foods with more moisture (> 40%).

This experiment was planned and performed to investigate the influence of formic acid as the conservant on the proteolysis degree and fermentation in lucerne silages.

Material and Methods

Lucerne of the cultivar NS Medijana ZMS V, of the second cut at the beginning of flowering was used. Ensiling was done in plastic barrels with the volume of 60 dm^3. After filling and compressing of the material, the samples for basic parameters of chemical composition and quality assessment were taken. The experiment was done as randomised design with three treatments (different doses of formic acid: 0; 3; 5 and 7 g/kg green lucerne mass) to investigate proteolysis and fermentation intensity.

When the fermentation ended, samples were taken and parameters of chemical composition, quality and proteolysis were determined (AOAC, 1984). Silage quality was determined according to Dulphy and Demarquilly (1981). Statistical analysis of obtained results was done by analysis of variance and significance testing (Snedecor and Cochran, 1982).

Results and Discussion

For all parameters of chemical composition of silages and energy values, it is confirmed that the change in the dose of conservant used had significant influence ($P<0.05$). The significantly higher dry matter content in treatments compared to control silage can be explained by the influence of formic acid addition, which is part of the organic and also dry matter (Table 1). Because of the formic acid addition the change in the relative content of certain chemical parameters occurred and their ration, which in most cases was significant. The increase of the conservant dose increased the amount of H^+ ions, which reduced proteolysis. Formic acid as organic matter and conservant increased net energy value of silages.

Tab. 1. - Chemical composition of silages

Parameters	Formic acid dose, g/kg green mass				Significance
	0	3	5	7	
DM, g/kg	220.18 b	236.61 a	235.41 a	231.63 a	*
g/kg DM:					
Crude protein	195.21 b	199.13 a	194.86 b	192.40 c	*
Protein nitrogen, g/kg N	248.22 c	394.55 b	391.55 b	518.79 a	**
Crude lipids	65.26	64.77	62.05	69.65	NS
Crude fibre	284.94 a	272.73 b	237.72 c	265.57 b	*
NFE	326.24 d	343.88 c	389.78 a	358.83 b	*
Ash	128.36 a	119.50 b	115.59 b	113.56 b	*
NEL (MJ)	4.87 b	4.94 ab	4.98 a	5.01 a	*
NEM (MJ)	4.65 b	4.72 ab	4.79 a	4.80 a	*

[a,b,c] Values in the same row with different letter are significantly different ($P<0.05$)

The content of certain amino acids was very variable in silages, which can be explained by the degradation processes and even by biosynthesis. The least preserved (compared with fresh lucerne) were following amino acids: histidine, threonine, arginine, serine, tyrosine (Table 2). The amounts of asparagic and glutamic acid, glycine and alanine were even higher in some treatments. McDonald (1981) explained that during the ensiling process glycine is a very common amino acid, and he explains the possibility of the increase in alanine content. He explained that the increased amount of proline is due to proline synthesis from glutamic acid. At the same time arginine and ornitine may be starting substances for proline synthesis. Proline has significant role in

detoxication, in the process of ammonia binding, while glutamic acid is renewed in the process of amination of α-ketoglutaric acid.

T a b. 2. - Amino acid composition of silages

Amino acids, g/kg N		Formic acid dose, g/kg green mass			
	Fresh lucerne	0	3	5	7
Lysine	6.00	4.02	6.05	3.94	2.61
Histidine	2.25	1.68	0.94	1.00	1.61
Fenilalanine	5.65	4.14	3.67	3.06	2.98
Leucine	8.29	6.77	7.70	4.92	6.24
Izoleucine	5.57	4.70	4.46	2.65	4.03
Threonine	5.50	1.50	3.96	2.59	4.54
Valine	6.11	5.47	6.05	4.96	5.50
Arginine	6.04	1.88	2.50	4.20	4.29
Asparagic acid	13.64	3.76	14.24	7.88	13.51
Serine	4.93	1.60	3.36	2.19	3.86
Glutamic acid	9.25	4.89	10.42	6.20	13.64
Proline	6.98	5.78	5.88	4.74	5.03
Glycine	4.96	4.33	5.39	2.88	4.82
Alanine	5.92	6.74	6.57	4.17	6.89
Tyrozine	3.83	1.42	1.16	0.56	1.11

The pH value of lucerne silage without conservant added was high (Table 3) and characteristic of this plant species. With the increase of formic acid dose the decrease in pH value is obtained. The degree of acidity was a result of dissociation of the produced lactic and acetic acid and also of the conservant used. In treated silages with medium and higher conservant dose the production of lactic acid was significantly higher, while silages with maximum conservant dose had significantly lesser free and bonded acetic acid. The presence of butyric acid in bonded form only in control silage had no statistical significance.

The amount of ammonia nitrogen compared to total N was first and basic confirmation of the protein degradation. In all treatments, as a result of proteolysis limitation, the amount of ammonia nitrogen was significantly decreased. The presence of ammonia in silages without butyric acid is explained by the activity of plant enzymes (McDonald, 1981). The same trend had the amount of soluble nitrogen. Silages with the formic acid added had ammonia and soluble nitrogen below permitted value for good quality (80-120 g/kg N for ammonia N and 500–600 g/kg N for soluble N; Dulphy and Demarquilly, 1981).

For silage quality evaluation the method of Dulphy and Demarquilly (1981) was used, which is taking into account quantity of acetic and butyric acid, ammonia and soluble nitrogen. According to this method, silage without formic

acid added and with the lowest dose were graded as class III, while silages with medium and high formic acid dose were graded as class II (Table 3).

T a b. 3. - Biochemical changes in lucerne silages

Parameters	Formic acid dose, g/kg green mass				Significance
	0	3	5	7	
PH	4.97 a	4.68 b	4.51 c	4.38 d	**
g/kg DM:					
Lactic acid	43.92 b	35.37 c	61.87 a	56.52 a	*
Acetic acid					
Free	28.19 a	32.95 a	29.60 a	22.35 b	*
Bonded	52.57	55.84	56.73	52.59	NS
Total	80.76 ab	88.79 a	86.33 a	74.94 b	*
Butyric acid					
Free	0.00	0.00	0.00	0.00	NS
Bonded	0.69	0.00	0.00	0.00	NS
Total	0.69	0.00	0.00	0.00	NS
Lactic acid / total acids	0.35	0.28	0.42	0.43	
g/kg N:					
Ammonia N / total N	136.92 a	109.86 b	71.74 c	46.02 d	**
Soluble N / total N	722.75 a	625.56 b	593.61 c	518.55 d	**
Quality class (Dulphy and Demarquilly, 1981)	III	III	II	II	

[a,b,c] Values in the same row with different letter are significantly different ($P<0.05$)

Conclusion

The use of formic acid as a conservant had significant influence on reduction of proteolytic processes in ensiling lucerne. Along with the increase of the conservant dose the decrease of pH value, amount of ammonia and soluble nitrogen occurred, while preservation of protein was enhanced. The amounts of amino acids varied because of degradation and biosynthesis. The most favorable ratio of lactic to total acids was found in silages with medium and high conservant dose. Silages with formic acid added had no butyric acid, while amounts of ammonia and soluble nitrogen were below the limiting value for good quality silages.

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Received May 10, 2005
Accepted November 15, 2005

EFEKTI UPOTREBE RAZLIČITIH DOZA MRAVLJE KISELINE KAO KONZERVANSA PRI SILIRANJU LUCERKE

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Rezime

U eksperimentu je ispitivan uticaj mravlje kiseline, korišćene u tri doze (3, 5 i 7 g/kg zelene mase) na intenzitet proteolize i fermentacije u silažama lucerke. Na osnovu rezultata hemijskih analiza utvrdjeno je da je sa porastom doze konzervansa došlo do snižavanja pH vrednosti, ograničavanja proteolize i povećanja očuvanosti proteina. Sadržaj pojedinih aminokiselina je dosta varirao usled njihovog razlaganja ili biosinteze. U tretiranim silažama utvrdjena je

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Rad je realizovan u okviru projekta broj TR-6813B koji je finansiran od strane Ministarstva nauke i zaštite životne sredine.

statistički značajno veća produkcija mlečne kiseline, dok su sulaže sa maksimalnom dozom konzervansa sadržale signifikantno manje slobodne i ukupne sirćetne kiseline. Prisustvo buterne kiseline u vezanom obliku samo u kontrolnoj silaži nije imalo statističku značajnost. Po metodi Dulphy-a i Demarquilly-a (1981) kontrolna i silaža sa minimalnom dozom konzervansa ocenjene su III klasom, a silaže sa dodatkom srednje i više doze mravlje kiseline II klasom kvaliteta.

Primljeno 10. maja 2005.
Odobreno 15. novembra 2005.