THE EFFECT OF LACTIC ACID ADDITIVE ON THE QUALITY AND CHEMICAL COMPOSITION OF MEADOW GRASS SILAGE¹

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Abstract: In the experiment, forage from the 1st cut of meadow grasses mown at the heading stage of dominant grasses was ensiled. The control silages were made from fresh forage (dry matter 223.5 g·kg⁻¹) and wilted forage (dry matter 351.9 g·kg⁻¹). The experimental silages were made from fresh forages supplemented with a 5% water solution of lactic acid (0.5 and 0.7 1·100 kg⁻¹ of forage) and 8% water solution of lactic acid (0.4 and 0.6 1·100 kg⁻¹ of forage). The highest contents of crude protein, water-soluble carbohydrates and energy were found in silages supplemented with 5% lactic acid applied at 0.7 1·100 kg⁻¹ of forage and in silages supplemented with 8% lactic acid applied at 0.6 1·100 kg⁻¹ of forage. The above silage variants were also characterized by the lowest content of NH₃-N in total-N, butyric acid and acetic acid, as well as the highest contents of lactic acid and the best indicators of fermentation quality. No important differences were found between silages made from wilted forage and silages made with lactic acid supplement.

Key words: meadow grass, silage, lactic acid additive, quality of fermentation, nutritive value

Introduction

Lactic acid, produced by lactic acid bacteria found on plants, is the main preservative of ensiled forage biomass. The intensity of lactic acid fermentation can be increased by supplementing the ensiled forages with preparations containing bacterial strains, mainly the *Lactobacillus* species. Rapid acidification of the ensiled material is important as it inhibits the growth of mould and putrefactive bacteria and the intensity of acetic acid, butyric acid and alcohol fermentation (Merry et al., 1995).

Watson and Nash (1971) presented the results of research Gerlach et al. (1929) in which the supplement of 1% and 2% lactic acid to the ensiled forage led to a rapid decrease in pH and a considerable reduction in undesirable strains of bacteria while preserving the populations of typical lactic acid bacteria in the ensiled biomass. However, the high costs of lactic acid production was a limiting factor in using it as a chemical additive to the ensiled forages.

¹ Original scientific paper - Originalni naučni rad

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Intensive development of the fermentation industry, which produces commercial quantities of lactic acid by fermentation of white sugar, enabled lactic acid to be used for ensiling purposes. In the European Union countries, lactic acid has been approved for enhancement of fermentation quality in the ensiled forages (*Haigh et al.*, 1996; *Pahlow and Honig*, 1996).

The experiments conducted in Poland demonstrated a positive effect of supplementing lactic acid to the ensiled legume (mainly red clover and alfalfa) forages on the course of fermentation and chemical composition of silages. In the experiments of Pyś (1997), Podkówka et al. (1998), Pyś and Pucek (1998), Ostrowski (1999), Pyś and Kański (2000) and Pyś et al. (2000), lactic acid supplemented to the ensiled red clover and fresh alfalfa silages had a significant effect on limiting the losses of crude protein and water soluble carbohydrates in the fermentation process. Silages made with lactic acid supplement were found to contain more lactic acid, little or no butyric acid and little ammonia-N compared to the control silages. Silages made with lactic acid supplement were also found to contain less butyric acid spores (Podkówka et al., 1998).

The principal ensiling material used in Poland in spring (May-June) is the 1st cut of meadow grasses, mown most often either prior to or at the heading stage. For high quality silages to be made from fresh meadow grasses, it is necessary to prewilt grasses in swaths or to use chemical additives as inhibitors of butyric or acetic fermentation or microbiological additives as stimulants of lactic acid fermentation in the ensiled forage biomass. Because prewilting of grasses in swaths is not always possible in the climatic conditions of Poland in May-June, the aim of the present experiment was to determine the effect of supplementing lactic acid to the ensiled fresh meadow grass forage on fermentation quality and chemical composition of silages.

Material and methods

The ensiling material used in the experiment was the 1st cut of meadow forage mown at the heading stage of dominant grasses. The proportion of meadow grasses (meadow fescue, perennial ryegrass and Italian ryegrass) in the meadow sward was 85%, that of legumes (white clover) 11% and forage herbs 4%. The level of mineral fertilization on a meadow moor in the vicinity of Cracow was 120 kg N·ha⁻¹, 100 kg P_2O_5 ·ha⁻¹ and 70 kg K_2O ·ha⁻¹, respectively.

Meadow forage was mown with a rotary mower and then cut into chaff 30-35 mm long using a drum cutter. In the experiment, fresh forage (dry matter 223.5 g·kg⁻¹) without additives (control silage WA) and wilted forage (dry matter 351.9 g·kg⁻¹), after 24 h prewilting in swaths) without additives (control silage WS) were ensiled. Wilted forage was also cut into chaff 30-35 mm long. The experimental silages were made from fresh forages supplemented with a 5% water solution of lactic acid applied at 0.5 1·100 kg⁻¹ of forage (5-LA5) and 0.7 1·100 kg⁻¹ of forage (7-LA5) and 8% water solution of lactic acid applied at 0.4 1·100 kg⁻¹ of forage (4-LA8) and 0.6 1·100 kg⁻¹ of forage (6-LA8). 50% lactic acid used in the experiment (PPF "Akwawit" Leszno, Poland) was characterized by specific weight of max. 1.16 g·ml⁻¹, total acidity min. 49.5%, water acidity min. 45%, lactic acid anhydride max. 5.0%, crude ash content max. 1.6%. Lactic acid fulfilled the requirements of the Polish Standard PN-76/A-86060.

Directly after comminution, unsupplemented forages were compacted in plastic silos 120 litres in volume. For the experimental silages, the weighted amount of comminuted forage was spread over polyethylene and subjected to ultra low volume spraying with different quantities and concentrations of a water solution of lactic acid. The whole was thoroughly mixed and compacted in silos 120 litres in volume. The silos were sealed such that excess gaseous products of fermentation were released and atmospheric air was prevented from entering. The silos were kept indoors at 16 ± 3 °C for 60 days. All silage variants were made in six replications.

Fresh forage samples taken directly after mowing, wilted forage samples taken directly after prewilting in swaths, and forage samples taken immediately after the tanks were opened were analysed by the drying method for the content of dry matter at 105 °C for 12 hours. The level of dry matter in silages was adjusted for volatile substances (Dulphy and Demarquilly, 1981). Buffer capacity of meadow forage was determined with the method of Playne and McDonald (1966). Forage and silage samples designed for further chemical analyses were dried in a blast drier at 50 °C for 72 hours and then ground. These samples were assayed for the contents of crude protein (% of total-N × 6.25, Kieldahl method), crude fibre and crude ash (AOAC, 1990) and ammonia-N (Skulmowski, 1974). The water soluble carbohydrate (WSC) content of forages and silages was determined colorimetrically (Dubois et al., 1956), NDF and ADF according to Van Soest et al. (1991) and gross energy in a bomb calorimeter ZK-10. The water extracts of silages were measured for pH. Silage samples taken directly after opening the silos and designed for the determination of organic acids were mixed for 1 minute with distilled water in the 1:5 ratio and then drained through a drain Filtrak 392. To 5 ml of the filtrate was added 1 ml of 25% solution of meta-phosphoric acid and after mixing centrifuged for 10 minutes (rev. 6000/min) and then decanted. These solutions were assayed for the contents of lactic, acetic and butyric acids by gas chromatography (calibration by the external standard method) using Varian 3400 CX with FID detector and column DB-FFAP (30 mm long, 0.540 mm across, filter thickness 1 µm) using argon as carrier gas.

The results were analysed statistically by evaluating the significance of differences among the parameters. Differences between measures of fermentation and nutrient content of the silages were estimated with one-factorial analysis of variance and Tukey's test using Statgraphic packet ver. 6.0.

Results and Discussion

The suitability of forages for ensiling is determined by their crude protein and WSC contents and buffer capacity. In the ensiled fresh meadow grass forage, crude protein was 152.3 g·kg⁻¹DM, WSC 123.9 g·kg⁻¹DM and buffer capacity 45.5 meq·100 g⁻¹DM (Table 1). The increased dry matter content of forages subjected to prewilting decreases buffer capacity and increases the WSC to buffer capacity ratio (*Yan et al., 1996*). Such an effect of prewilting meadow grass forage was observed in the present experiment. The increased dry matter of wilted forages was accompanied by increased WSC concentration (130.8 g·kg⁻¹DM) and decreased buffer capacity (34.8 meq·100 g⁻¹DM) (Table 1).

Item - pokazatelj	Meadow grass-Livadske trave			
item - pokazaterj	Fresh-sveža	Wilted-provenuta		
Dry matter –suva materija g·kg ⁻¹	223.5	351.9		
	g·kg ⁻¹ DM			
Organic matter- organska materija	913.3	917.5		
Crude protein-sirovi protein	152.3	148.5		
Crude fibre-celuloza	225.6	230.7		
NDF ¹	689.5	660.7		
ADF ²	319.7	287.6		
WSC ³	123.9	130.8		
Crude ash – pepeo	86.7	92.5		
Gross energy –ukupna energija, MJ·kg ⁻¹ DM	17.6	18.5		
Buffer capacity-puferni kapacitet, meq·100 g ⁻¹ DM	45.5	34.8		

Table 1. Chemical composition of meadow grass forages before ensiling Tabela 1. Hemijski sastav smeše livadskih trava pre siliranja

The nutrient content of meadow grass silages is shown in Table 2. The dry matter content of silages without additives (WA) was 199.9 g·kg⁻¹. The lower content of dry matter in these silages compared to forages before ensiling pointed to great losses of this component in the process of fermentation. The supplement of lactic acid to the ensiled forage caused dry matter in silages to increase (P<0.01) compared to WA silages. The concentration and quantity of the acid applied was found to have no significant effect on the dry matter content (212.7–220.2 g·kg⁻¹) of silages made with its addition. The beneficial effect of lactic acid supplement on the dry matter content of fresh meadow grass silages was also reported by *Ostrowski* (2000).

In the present experiment, lactic acid supplement did not have a significant effect on the content of crude fibre and its NDF and ADF fractions in the silages. Lower NDF contents (by 9.5–14.8 g·kg⁻¹DM on average) of the silages supplemented with lactic acid compared to WA silages could result more from chemical changes during the fermentation of the ensiled material than from the direct effect of the same lactic acid as a chemical additive.

Ensiling fresh meadow grass forage without additives (WA) led to great crude protein losses in the process of fermentation. The level of crude protein in WA silages was 128.9 g·kg⁻¹DM. The supplement of lactic acid to the ensiled forage led to a significant (P<0.01) inhibition of protein degradation in silages 5-LA5, 7-LA5, 4-LA8 and 6-LA8 in which the content of this component was 139.2, 142.9, 141.7 and 144.1 g·kg⁻¹DM, respectively. A similar favourable effect on limiting crude protein losses in the fermentation process was exerted by prewilting of forages prior to ensiling. The crude protein content of WS silages was 146.0 g·kg⁻¹DM.

¹ neutral-detergent fibre – celuloza u neutralnom deterdžentu

² acid-detergent fibre – celuloza u kiselom deterdžentu

³ water-soluble carbohydrates – ugljeni hidrati rastvorljivi u vodi

	Type of silage – tip silaže					SEM-	
Item - pokazatelj	WA	WS	5-LA5	7-LA5	4-LA8	6-LA8	stand. greška
Dry matter – suva materija	199.9 °	346.5 a	212.7 b	215.0 ^b	219.2 b	220.2 b	54.82
	g·kg ⁻¹ DM						
Organic matter-organska mater.	915.7	907.1	913.7	913.1	911.2	909.0	3.18
Crude protein-sirovi protein	128.9 ^b	146.0 a	139.2 ^a	142.9 ^a	141.7 ^a	144.1 ^a	6.57
Crude fibre-celuloza	213.3	215.2	212.6	212.9	213.5	212.6	0.98
NDF ¹	599.2 ^a	571.2 b	589.7 °	586.3 °	585.8 °	584.0 °	9.07
ADF ²	302.1	293.3	307.5	306.9	304.5	303.6	5.16
WSC ³	88.6 ^d	99.7 ^{tx}	104.5 ^{to}	109.5 ab	107.8 ab	114.5 ^a	8.97
Crude ash – pepeo	84.3	92.9	86.3	86.9	88.8	90.1	3.05
Gross energy-ukupna energija MJ·kg ⁻¹ DM	15.2	16.3	16.8	16.9	16.6	17.3	0.72

Table 2. Nutrients content in meadow grass silages Tabela 2. Sadržaj hranljivih materija u silaži smeše livadskih trava

Različita slova iznad proseka u istom redu označavaju statistički značajnu razliku (P < 0.01)

The greatest differences in the chemical composition of silages concerned the WSC content. The greatest WSC losses during the fermentation occurred in WA silages in which WSC content was 88.6 g·kg⁻¹DM. In wilted forage silages (WS), the content of WSC was higher (P<0.01) by an average of 11.1 g·kg⁻¹DM. The supplement of 5% lactic acid greatly limited the losses of WSC, the content of which was higher (P<0.01) by an average of 15.9 g·kg⁻¹DM in 5-LA5 silages and by an average of 20.9 g·kg⁻¹DM in 7-LA5 silages compared to silages without additives (WA). WSC losses were most efficiently reduced in the fermentation process by using a 8% lactic acid supplement to the ensiled forage. Compared to WA silages, WSC content of 4-LA8 silages was higher (P<0.01) by an average of 19.2 g·kg⁻¹DM and in 6-LA8 silages by 25.9 g·kg⁻¹DM on average.

The high WSC content of silages supplemented with lactic acid, especially 8% lactic acid, demonstrated that the lactic acid supplement to the ensiled forage biomass may limit the use of WSC as a sugar substrate by lactic acid bacteria while adding to the content of lactic acid produced by these bacteria in the fermentation process. In silage making, limiting WSC losses is desirable because decreased WSC content of silages is accompanied by decreasing amounts of readily available energy to animals fed with such feeds (McDonald et al., 1991).

^{1, 2, 3} Designations as in Table 1. – označeno kao u tab. 1.

WA - without additive - bez aditiva

WS - wilted silage - provenuta silaža

 $^{5\}text{-LA5}$ – with 5% lactic acid additive (0.5 l·100 kg⁻¹ of fresh forage) - sa 5% aditiva mlečne kiseline (0.5 l na 100 kg^{-1} sveže silaže)

⁷⁻LA5 – with 5% lactic acid additive (0.7 l·100 kg⁻¹ of fresh forage)

⁴⁻LA8 – with 8% lactic acid additive (0.4 l·100 kg⁻¹ of fresh forage)

⁶⁻LA8 – with 8% lactic acid additive (0.6 l·100 kg⁻¹ of fresh forage)

SEM – standard error of means – standardna greška proseka

a, b, c, d Means with different letters in the same row differ significantly (P < 0.01)

The NH₃-N content, pH value and the content of organic acids in meadow grass silages are shown in Table 3. The unfavourable fermentation process in the ensiled forage without additives resulted in a high pH value of 4.77 in WA silages. The lactic acid supplement to the ensiled forage had a beneficial effect on reaching pH value in the 4.18 – 4.32 range which is considered optimal for grass silages made with chemical supplements (McDonald et al., 1991; Ostrowski, 1999; Brzóska et al., 1999).

Table 3. NH₃-N and organic acids content in meadow grass silages*

Tabela 3. Sadržaj amonijačnog	u ukupnom azotu i organskih kilseina	a u silaži smeše livadskih trava

Item - pokazatelj	Type of silage – tip silaže					SEM	
nem - pokazatelj	WA	WS	5-LA5			6-LA8	SEN
pH	4.77 ^a	4.53 b	4.32 °	4.21 ^{cd}	4.24 ^{cd}	4.18 ^d	0.224
NH ₃ -N-amonijačni u ukupnom azotu, g·kg ⁻¹ of total-N	106.7 ª	58.5 ^b	50.8 °	44.7 °	47.7 °	40.9 ^d	24.49
Organic acids-organske kiseline:	g·kg ⁻¹ DM-S.M.						
Lactic-mlečna	65.9 b	71.6 a	72.0 a	76.7 a	73.9 ^a	78.3 ª	4.63
Acetic-sircetna	23.7 ^a	16.1 ^b	17.3 b	16.2 b	16.4 b	14.1 ^b	3.31
Butyric-buterna	8.6 a	4.6 ^b	4.4 ^b	3.4 bc	3.8 bc	2.8 °	2.06
Lactic acid % of total acids - mlečna kis. % od ukupnih kis.	67.1 ^b	77.6 ^a	76.8 ^a	79.6 ª	79.0 ^a	82.2 ^a	5.25
Lactic acid / acetic acid ratio - odnos mlečna/sirćetna kiselina	2.78 ^b	4.44 a	4.16 ^a	4.73 ^a	4.50 ^a	5.55 ^a	0.92

^{*} Designations as in table 2. – označeno kao u tabeli 2.

In the present experiment, the rate of protein degradation in the fermentation process was measured by the proportion of NH₃-N in total-N (g NH₃-N·kg⁻¹ of total-N). The highest NH₃-N content was characteristic of WA silages (106.7 g·kg⁻¹ of total-N). Ensiling wilted meadow grasses led to a significant (P<0.01) decrease in the proportion of NH₃-N in total-N of WS silages by 48.2 g on average. Most of the studies pointed to a beneficial effect of prewilting silages prior to ensiling on lowering the NH₃ content and decreasing the proportion of NH₃-N in total-N of silages (Haigh, 1990; Gasior and Brzóska, 2000). The present findings confirmed such an effect of prewilting forages.

The lactic acid supplement to the ensiled meadow grass forage caused a significant (P<0.01) decrease in the NH₃ content of silages. The proportion of NH₃-N in total-N of silages 5-LA5, 7-LA5, 4-LA8 and 6-LA8 was lower (P<0.01) by an average of 55.9, 62.0, 62.0 and 65.8 g respectively compared to WA silages. Considerable limitation in the amount of NH₃ produced during fermentation of the ensiled grass forages supplemented with lactic acid is in agreement with the data of *Podkówka et al.* (1998) and Ostrowski (2000). In the studies of Podkówka et al. (2000), silages made from field cultivated grasses and fresh meadow grasses without additives contained 200 and 170 g·kg⁻¹ of total-N, respectively. Ensiling these forages with a 5% lactic acid supplement brought the NH₃-N content of silages down to 90 g·kg⁻¹ of total-N.

Silages made from meadow grasses without additives (WA) were characterized by lower intensity of lactic acid fermentation compared to the other silages. In WA silages lactic acid content was 65.9 g·kg⁻¹DM, while in WS silages it was higher (P<0.01) by an average of 5.7 g·kg⁻¹DM. In 5-LA5 and 7-LA5 silages, lactic acid was higher (P<0.01) by an average of 6.1 and 10.8 g·kg⁻¹DM, and in 4-LA8 and 6-LA8 silages by 8.0 and 12.4 g·kg⁻¹DM on average compared to WA silages.

Similar trends were observed for the content of lactic and butyric acid in silages. The highest content of acetic acid (23.7 g·kg⁻¹DM) and butyric acid (8.6 g·kg⁻¹DM) was noted in WA silages. The acetic acid and butyric acid contents were lower (P<0.01) by 7.6 and 4.0 g·kg⁻¹DM in WS silages, by 6.4 and 4.2 g·kg⁻¹DM in 5-LA5 and 7-LA5 silages, and by 7.3 and 5.8 g·kg⁻¹DM in 4-LA8 and 6-LA8 silages. A similar limiting effect of lactic acid supplement to the ensiled fresh grass forages on the intensity of acetic and butyric acid fermentation was reported earlier by *Podkówka et al.* (1998) and Ostrowski (2000).

In the present studies it is worth noting a positive effect of the lactic acid supplement, especially 8% lactic acid, to the ensiled meadow grass forages on greatly inhibiting the intensity of butyric acid fermentation in the silages. Feeding silages with a high content of butyric acid leads to excessive amounts of it being stored in the rumen. The ruminal degradation of butyric acid requires much energy, which is a great loss for the organism of animals, especially high-yielding cows and goats (Barej, 1990; Morand-Fehr, 1991).

In the present experiment, the correctness of the fermentation process in the ensiled meadow grass forages was determined by two indicators: percentage proportion of lactic acid in total acids (LA/TA) and lactic acid to acetic acid ratio (LA/AA) (Table 3). In WA silages, LA/TA was 67.1% and LA/AA 2.78. In WS silages, LA/TA and LA/AA were higher (P<0.01) at 77.6% and 4.44 respectively. The lactic acid supplement to the ensiled meadow grass forage limited the intensity of acetic and butyric acid fermentation, as reflected in the values of indicators. In silages made with 5% lactic acid supplement, LA/TA was 76.8% and 79.6% and LA/AA 4.16 and 4.73. The supplement of 8% lactic acid to the ensiled forage further increased the value of LA/TA (79.0% and 82.2%) and LA/AA (4.50 and 5.55). A similar tendency towards increased percentage proportion of lactic acid in total acids and towards increased lactic acid to acetic acid ratio in meadow grass and field cultivated grass silages was reported by Podkówka et al. (1998) and Ostrowski (2000).

Conclusions

Lactic acid used in the present experiment to supplement the ensiled fresh meadow grass forage is considered a good chemical preparation. Silages made with this supplement, especially 5% lactic acid applied at 0.7 1·100 kg⁻¹ of forage and 8% lactic acid applied at 0.6 1.100 kg⁻¹ of forage were characterized by the highest contents of crude protein, water soluble carbohydrates and energy. 8% lactic acid applied at 0.6 1.100 kg⁻¹ of forage was the most efficient in limiting the degradation of proteins and was an adequate inhibitor of butyric and acetic acid fermentation in the ensiled biomass of meadow grass forages. In the present study, no significant differences were found between silages made from wilted forage and silages made from fresh meadow grasses supplemented with lactic acid, in terms of the contents of crude protein and energy and parameters of fermentation quality. It is therefore concluded that under favourable atmospheric conditions, prewilting of forages in swaths prior to ensiling continues to ensure the high quality of silages. Where prewilting of meadow grass or field cultivated grass forages is not possible, it is recommended to ensile these forages with the chemical supplement of lactic acid, ensuring silages characterized by low protein and WSC losses and high quality of fermentation in the ensiled biomass.

UTICAJ DODAVANJA MLEČNE KISELINE NA KVALITET I HEMIJSKI SASTAV SILAŽE LIVADSKIH TRAVA

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Rezime

U istraživanjima korišćena mlečna kiselina kao dodatak silaži od smeše livadskih trava (livadski vijuk, engleski ljulj i italijanski ljulj ukupno čine 85%, bele deteline 11% i ostalih krmnih vrsta 4%) se pokazala kao dobar hemijski preparat. Spravljena silaža sa ovim dodatkom naročito 5% dodate mlečne kiseline sa 0,7 l x 100 kg-1 mase i 8% mlečne kiseline dodavane 0,6 l x 100 kg-1 mase se karakterisala najvišim sadržajem sirovih proteina, ugljenih hidrata rastvorljivih u vodi i energijom. Dodatak 8% mlečne kiseline u količini 0,6 l x 100 kg-1 mase je bilo najefikasnije u ograničavanju razlaganja proteina i pokazalo se kao povoljan inhibitor fermentiranja buterne i sirćetne kiseline u siliranoj biomasi smeše livadskih trava. U našem istraživanju nisu uočene značajne razlike između silaža spravljanih od provenute mase i silaže spravljane od sveže pokošene mase dopunjavane mlečnom kiselinom u pogledu sadržaja sirovih proteina, energije kao i pokazatelja kvaliteta fermentacije.

Iz svega navedenog zaključuje se da, u povoljnim atmosferskim uslovima, provenuta pokošena masa pre siliranja nastavlja da zadržava visok kvalitet silaže. Tamo gde nije moguće provenjavanje smeše livadskih trava ili gajenih trava, preporučuje se da se silira masa sa dodatkom mlečne kiseline, pri čemu se dobijena silaža odlikuje niskim gubicima proteina i ugljenih hidrata rastvorljivih u vodi i visokim kvalitetom fermentacije silirane biomase.

Ključne reči: livadska trava, silaža, aditiv od mlečne kiseline, kvalitet fermentacije, nutritivna vrednost

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