

## EFFECT OF LACTIC ACID BACTERIAL INOCULANT WITH ENZYME AND ROLLED BARLEY ADDITIVE ON THE CHEMICAL COMPOSITION AND PROTEIN DEGRADATION OF ALFALFA SILAGE<sup>1</sup>

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**Abstract:** In the experiment, forage (dry matter 216.3 g.kg<sup>-1</sup>) from the 1st cut of alfalfa (*Medicago sativa* L.) cv, Radius, mown at the early flowering stage, was ensiled. Alfalfa was ensiled without inoculants; with lactic acid bacteria and enzyme inoculants; with rolled barley; or with rolled barley and lactic acid bacteria and enzyme inoculants. Rolled barley was added in amounts of 50, 100, 150 and 200 g.kg<sup>-1</sup> of alfalfa forage. The bacterial acid enzyme inoculants (Bactozym) contained cultures of *Lacto bacillus casein*, *Enterococcus faecium* M74 and *Pediococcus sp.* (a total of  $1.5 \times 10^9$  CFU in 1g of friable preparation) in the powdered part and the cellulase-hemicellulase complex (25000 n cat. in 1ml) and glucose-oxidase (4000 n cat. in 1ml) in the liquid part. The inoculants was used in a water solution in the amount of 10mg bacterial component and 0,2 ml enzymatic component / kg<sup>-1</sup> alfalfa forage. The supplement of only rolled barley to alfalfa forage was found to have no significant effect on improving the processes of proteolysis and deamination in this type of silages, compared to alfalfa silages made with bacterial and enzyme inoculants. Significantly lower crude protein and WSC losses in the fermentation process, increased intensity of lactic acid fermentation, the lack of propionic and butyric acid fermentation, and significantly reduced processes of protein degradation were found in alfalfa-rolled barley silages made with the addition of lactic acid bacteria and enzyme inoculants.

**Key words:** alfalfa, silage, lactic acid bacteria and enzyme inoculants, effluent absorbent, quality of fermentation, protein degradation

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## Introduction

Alfalfa (*Medicago sativa* L.) is ranked among hard-to-ensile plants due to its high content of crude protein and low content of water-soluble carbohydrates (WSC), which are responsible for increased buffer capacity (Podk6wka *et al.*, 1995). The fermentation process and ensilability increase as a result of prewilting alfalfa forage or using ensilage additives in the form of fermentation inhibitors or stimulants (McDonald *et al.*, 1991). Where prewilting of alfalfa is not possible, the addition of effluent absorbents may help to minimize seepage of effluents and improve silage quality (Offer *et al.*, 1991). Ground grain, bran, dried sugar beet pulp and straw can be used as absorbents (O'Kiely, 1990; Ferns and Mayne, 1994a, 1994b). The fermentation process in the ensiled alfalfa is accompanied by intensive protein degradation (Fairbairn *et al.*, 1992). Protein degradation results from the high activity of cellular proteolytic enzymes after the life processes of plants come to a halt and from the process of bacterial deamination of free amino acids and amides, which is caused by fermentation bacteria, especially *Clostridium* species (Makoni *et al.*, 1997; Vagnoni *et al.*, 1997). The degradation of alfalfa protein both after the forage has been cut and in the silo leads to a high protein degradation in the rumen. This depresses digestion, reduces the synthesis of microbiological protein in the rumen and decreases the amount of amino acids flowing from the rumen to the duodenum in cattle and sheep (Tamminga *et al.*, 1991). Cellular proteolysis of protein can be efficiently limited by rapidly prewilting forage, bringing pH down to 4.0, and thoroughly compacting the forage to prevent aerobic processes and an increase of temperature in the silo (Charmley and Veira, 1990).

Silage making with effluent absorbents is an alternative to prewilting alfalfa forage under natural conditions prior to ensiling. The use of absorbents considerably inhibits the seepage of effluent, reduces losses and improves fermentation quality in the silo and the nutritive value of silages. Absorbents in the form of ground grain or dried sugar beet pulp decrease pH value and the acetic acid content of silages and elevate the concentration of lactic acid and water soluble carbohydrates in the silage (Harrison *et al.*, 1994; Moore and Kenedy, 1994; Rouzbehan *et al.*, 1996).

The aim of the present research was to determine the effects of a lactic acid bacteria and enzyme inoculants and effluent absorbents in the form of rolled barley grain on fermentation intensity and degree of protein degradation in alfalfa silages.

## Material and Methods

In the experiment, forage from the 1st cut of alfalfa (*Medicago sativa* L.) cv. Radius, mown at the early flowering stage (16-17 June 1999) was used for ensiling. Immediately after being cut by a rotary mower, forage (dry matter 216.3 g·kg<sup>-1</sup>) was cut into chaff 25-30 mm long using a drum cutter. The following experimental silages were made: alfalfa without additives (WA.); with absorbent in the form of rolled barley grain amounts of 50 g·kg<sup>-1</sup> forage (B1), 100 g·kg<sup>-1</sup> forage (B2), 150 g·kg<sup>-1</sup> forage (B3) and 200 g·kg<sup>-1</sup> forage (B4); with bacteria and enzyme inoculants (BE); with bacteria and enzyme inoculants and rolled barley grain in the amounts of 50 g·kg<sup>-1</sup> forage (BE-B1), 100 g·kg<sup>-1</sup> forage (BE-B2), 150 g·kg<sup>-1</sup> forage (RF.-R3) and 200 g·kg<sup>-1</sup> forage (RE-R4).

Comminuted alfalfa was spread over polyethylene sheeting, sprinkled with predetermined amounts of the absorbent, mixed and compacted in silos. In the bacteria and enzyme inoculants variant, the alfalfa and absorbent mixture was sprayed with a water solution of the experimental inoculants and thoroughly mixed and compacted in plastic 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. All silage variants were made in four replications. The silages were stored indoors for 60 days at  $17 \pm 2$  °C.

The lactic acid bacteria and enzyme inoculants Bactozyme (Medipharm CS GmbH, Hustopece -Brno, Czech Republic) used in the experiment contained cultures of *Lactobacillus plantarum*, *Lactobacillus casein*, *Enterococcus faecium* M-74 and *Pediococcus sp.* (a total of  $15 \times 10^9$  CFU in 1 g of friable preparation) in the powdered part and the cellulase-hemicellulase complex (25000 n cat. in 1 ml) and glucose-oxidase (4000 n cat. in 1 ml) in the liquid part. The Bactozym inoculants was used in a water solution in the amount of 10 mg bacterial component and 0.2 ml enzymatic component /  $\text{kg}^{-1}$  alfalfa forage.

The dry matter content of forage samples, absorbents and silages was determined with the drying method at 105° C for 12 hours. The dry matter content of silages was adjusted for volatile components (*Dulphy and Demarquilly, 1981*). Buffer capacity of the alfalfa forage was determined according to *Playne and McDonald (1966)*. Forage absorbent 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 x 6.25), crude fibre (AOAC, 1990), ammonia-N (*Skulmowski, 1974*) and protein-N (*Licitra et al., 1996*). The content of water-soluble carbohydrates (WSC) in the forages and silages was determined colorimetrically (*Dubois et al. 1956*) while ADF and NDF following the method of *Van Soest et al. (1991)*. The water extracts of silages were measured for pH. Silage samples to be assayed for the content of organic acids were mixed for 1 minute with distilled water in the ratio 1:5 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 ( $t = 10$  min, 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 CD with FID detector and column DB-FFAP (30 mm long, 0.540 mm across, filter thickness 1  $\mu\text{m}$ ) using argon as carrier gas.

The results were analyzed statistically to estimate significant differences among the parameters studied, differences between measures of fermentation and protein degradation in the silages were estimated with one-factorial analysis of variance and Tukey's test using Statgraphic packet ver. 6.0.

### Results and Discussion

Chemical composition of alfalfa, rolled barley and alfalfa-rolled barley mixtures is shown in Table 1. The dry matter content of alfalfa was  $216.3 \text{ g}\cdot\text{kg}^{-1}$  and buffer capacity  $78.6 \text{ meq}\cdot 100\text{g}^{-1}$  DM. Alfalfa was characterized by a high level of crude protein of  $256.3 \text{ g}\cdot\text{kg}^{-1}$  DM in relation to the moderate WSC content of  $86.9 \text{ g}\cdot\text{kg}^{-1}$  DM. The rolled barley

supplements of 50, 10, 150 and 200 g•kg<sup>-1</sup> of alfalfa forage increased the dry matter content of alfalfa-rolled barley mixtures by 248.6 to 350.9 g•kg<sup>-1</sup>. As the proportion of rolled barley increased the crude protein content decreased and the WSC content of the mixtures increased steadily. The increased proportion of the absorbent also reduced crude fibre, NDF and ADF in the mixtures. Similar trends were observed in the studies of *Harrison et al.* (1994), where the addition of rolled barley in amounts of 50, 100 and 150 g•kg<sup>-1</sup> of alfalfa forage at dry matter content of 160, 240 and 320 g•kg<sup>-1</sup> increased the content of dry matter and starch and decreased crude protein, NDF and ADF in alfalfa-rolled barley mixtures before ensiling.

Table 1. Chemical composition (g•kg<sup>-1</sup>DM) of alfalfa, rolled barley, and alfalfa-rolled barley mixtures before ensiling

Tabela 1. Hemijski sastav (g•kg<sup>-1</sup>DM) lucerke, gnječenog ječma, i mešavine lucerke i gnječenog ječma pre siliranja

Item / Stavka	Alfalfa forage / Lucerka	Rolled barley / Gnječeni ječam	Level of added rolled barley g•kg <sup>-1</sup> of alfalfa forage / Nivo dodatog gnječenog ječma u g•kg <sup>-1</sup> lucerke			
			50	100	150	200
Dry matter / Suva materija g•kg <sup>-1</sup>	216.3	896.7	248.6	277.8	317.3	350.9
Crude protein / Sirovi protein	256.3	121.6	250.2	243.2	235.8	228.5
Crude fibre / Sirova vlakna	241.6	45.9	232.0	221.1	209.9	202.1
NDF <sup>1</sup>	495.3	221.0	483.6	470.5	455.1	442.3
ADF <sup>2</sup>	281.4	76.1	272.6	261.9	252.8	239.8
WSC <sup>3</sup>	86.9	109.6	88.8	90.3	91.5	92.6
Buffer capacity meq•100 g <sup>-1</sup> DM / Puferski kapacitet meq•100 g <sup>-1</sup> SM	78.6	nd	nd	nd	nd	nd

<sup>nd</sup> Not determined / nije određen

<sup>1</sup> Neutral-detergent fibre / vlakna u neutralnom deterdžentu

<sup>2</sup> Acid-detergent fibre / vlakna u biljnom materijalu

<sup>3</sup> Water-soluble carbohydrates / ugljeni hidrati rastvorljivi u vodi

Chemical composition of alfalfa and alfalfa-rolled barley silages is shown in Table 2. Alfalfa silages made without additives (WA) were characterized by the highest content of NDF (480.6 g•kg<sup>-1</sup>DM) and ADF (269.8 g•kg<sup>-1</sup>DM). Ensiling alfalfa with lactic acid bacteria and enzyme inoculants (BE) significantly ( $P < 0.01$ ) decreased NDF (419.2 g•kg<sup>-1</sup>DM) and ADF (223.1 g•kg<sup>-1</sup>DM) in silages. In silages (B1, B2, B3, B4) made from alfalfa supplemented with rolled barley, NDF content ranged from 465.3 to 436.2 g•kg<sup>-1</sup>DM and ADF content from 263.4 to 231.2 g•kg<sup>-1</sup>DM. Ensiling alfalfa-rolled barley mixtures with bacterial and enzyme inoculants further reduced the contents of NDF (413.2 – 422.1 g•kg<sup>-1</sup>DM) and ADF (218.1 – 226.0 g•kg<sup>-1</sup>DM) in silages BE-B1 – BE-B4. The lower NDF and ADF contents of these silages were due to the activity of cell-wall polysaccharide degrading enzymes in the ensiled matter. The present results are in agreement with the studies of *Selmer-Olsen et al.* (1993) and *Nadeau et al.* (1996) in

which silages made with enzyme preparations had a significantly lower content of fibre fractions compared to those made without enzymes. The lowest WSC content ( $37.3 \text{ g}\cdot\text{kg}^{-1}\text{DM}$ ) was found in alfalfa silages without additives (WA). In the silages with bacterial and enzyme inoculants (BE), WSC content was significantly ( $P<0.01$ ) higher at  $49.9 \text{ g}\cdot\text{kg}^{-1}\text{DM}$ . The water-soluble carbohydrates content of silages B1, B2, B3 and B4 was similar at 50.6, 51.8, 50.9 and  $52.1 \text{ g}\cdot\text{kg}^{-1}\text{DM}$  respectively. Significantly higher ( $P<0.01$ ) contents of WSC (65.2, 65.9, 64.8 and  $66.1 \text{ g}\cdot\text{kg}^{-1}\text{DM}$ ) were observed in silages BE-B1, BE-B2, BE-B3 and BE-B4. The low WSC content of WA silages resulted from its losses during the fermentation of alfalfa ensiled without additives. The much higher WSC content of BE silages was due to the activity of cell-wall degrading enzymes (Selmer-Olsen, 1993). The use of rolled barley as an effluent absorbent and an additional source of nutrients increased the WSC content of silages (Harrison *et al.*, 1994). This effect was confirmed in the present study for silages made with rolled barley. The highest WSC contents of BE-B1 – BE-B4 silages was due to both the rolled barley supplement and the activity of enzymes that increase WSC by hydrolyzing the polysaccharides of cell walls of plant components used for ensiling. One of the main components of barley grain is starch whose content in alfalfa-rolled barley mixtures increases with the increasing proportion of rolled barley in the mixture (Harrison *et al.*, 1994). In the fermentation process, starch hydrolysis under the influence of  $\beta$ -glucanase enzymes releases large quantities of simple sugars, mainly glucose (Jones, 1988). The high level of WSC in alfalfa-rolled barley silages made with the addition of lactic acid bacteria and enzyme inoculants resulted from the combined effects of the enzymatic hydrolysis of rolled barley starch, the hydrolysis of alfalfa cell wall polysaccharides, and the better quality of fermentation in these silages, leading to low WSC losses.

In the present experiment, the lowest fermentation quality was characteristic of alfalfa silages made without additives (WA). These silages had the lowest content of lactic acid ( $43.3 \text{ g}\cdot\text{kg}^{-1}\text{DM}$ ) and the highest contents of acetic acid ( $34.8 \text{ g}\cdot\text{kg}^{-1}\text{DM}$ ), propionic acid ( $1.2 \text{ g}\cdot\text{kg}^{-1}\text{DM}$ ) and butyric acid ( $8.8 \text{ g}\cdot\text{kg}^{-1}\text{DM}$ ). The highest content of lactic acid ( $66.8 \text{ g}\cdot\text{kg}^{-1}\text{DM}$ ), considerable reductions in acetic acid ( $26.1 \text{ g}\cdot\text{kg}^{-1}\text{DM}$ ), propionic acid ( $0.5 \text{ g}\cdot\text{kg}^{-1}\text{DM}$ ), butyric acid ( $2.6 \text{ g}\cdot\text{kg}^{-1}\text{DM}$ ) and pH reduced to 4.68 were found in the silages (BE) with bacterial and enzyme inoculants. Increased intensity of lactic fermentation and reduced pH in grass or alfalfa silages made with bacterial and enzyme inoculants were also observed by Rooke *et al.* (1990), Harrison *et al.* (1994) and Sharp *et al.* (1994).

Ensiling alfalfa with the supplement of rolled barley alone did not increase the intensity of lactic fermentation. In B1 – B4 silages, lactic acid decreased as rolled barley increased, being lower ( $P<0.01$ ) by an average of  $14.2 - 16.5 \text{ g}\cdot\text{kg}^{-1}\text{DM}$  compared to BE silages. The supplement of rolled barley to the ensiled alfalfa significantly ( $P<0.01$ ) inhibited propionic and butyric acid fermentation in the silages. In B1 – B4 silages, the propionic acid content was  $0.2 - 0.4 \text{ g}\cdot\text{kg}^{-1}\text{DM}$  and that of butyric acid  $0.9 - 1.4 \text{ g}\cdot\text{kg}^{-1}\text{DM}$ . The best fermentation quality was characteristic of alfalfa-rolled barley silages made with bacterial and enzyme inoculants. In BE-B1, BE-B2, BE-B3 and BE-B4 silages, the lactic acid content was 64.3, 65.9, 67.3 and  $61.9 \text{ g}\cdot\text{kg}^{-1}\text{DM}$  and that of acetic acid 23.3, 23.0, 22.4 and  $22.6 \text{ g}\cdot\text{kg}^{-1}\text{DM}$  respectively. No propionic acid and butyric acid was found in these silages.

Table 2. Chemical composition ( $\text{g}\cdot\text{kg}^{-1}\text{DM}$ ) of alfalfa and alfalfa-rolled barley silages made with or without addition of a lactic acid bacteria and enzyme inoculant

Tabela 2. Hemijski sastav ( $\text{g}\cdot\text{kg}^{-1}\text{SM}$ ) lucerke i silaže od lucerke i gnječnog ječma sa ili bez dodavanja bakterije mlečne kiseline i enzimskog inokulanta

Item	Type of silage / Tip silaže										SEM
	WA	BE	B1	B2	B3	B4	BE-B1	BE-B1	BE-B3	BE-B4	
Dry matter / Suva materija, $\text{g}\cdot\text{kg}^{-1}$	191.1 <sup>e</sup>	200.3 <sup>e</sup>	238.6 <sup>d</sup>	270.3 <sup>c</sup>	311.8 <sup>b</sup>	346.3 <sup>a</sup>	234.2 <sup>d</sup>	266.3 <sup>c</sup>	307.5 <sup>b</sup>	343.8 <sup>a</sup>	4.23
Crude protein / Sirovi protein	200.9 <sup>b</sup>	217.5 <sup>ab</sup>	223.8 <sup>a</sup>	221.6 <sup>a</sup>	219.8 <sup>ab</sup>	211.6 <sup>b</sup>	232.1 <sup>a</sup>	229.8 <sup>a</sup>	223.0 <sup>a</sup>	216.9 <sup>ab</sup>	2.98
NDF <sup>1</sup>	480.6 <sup>a</sup>	419.2 <sup>d</sup>	465.3 <sup>b</sup>	456.8 <sup>b</sup>	441.8 <sup>c</sup>	436.2 <sup>c</sup>	422.1 <sup>d</sup>	420.6 <sup>d</sup>	409.3 <sup>d</sup>	413.2 <sup>d</sup>	5.34
ADF <sup>2</sup>	269.8 <sup>a</sup>	223.1 <sup>c</sup>	263.4 <sup>ab</sup>	250.0 <sup>ab</sup>	248.6 <sup>b</sup>	231.2 <sup>bc</sup>	226.0 <sup>c</sup>	221.1 <sup>c</sup>	219.8 <sup>c</sup>	218.1 <sup>c</sup>	3.16
WSC <sup>3</sup>	37.3 <sup>c</sup>	49.9 <sup>b</sup>	50.6 <sup>b</sup>	51.8 <sup>b</sup>	50.9 <sup>b</sup>	52.1 <sup>b</sup>	65.2 <sup>a</sup>	65.9 <sup>a</sup>	64.8 <sup>a</sup>	66.1 <sup>a</sup>	2.19
pH	5.31 <sup>a</sup>	4.68 <sup>b</sup>	4.46 <sup>c</sup>	4.44 <sup>c</sup>	4.43 <sup>c</sup>	4.40 <sup>c</sup>	4.26 <sup>a</sup>	4.23 <sup>a</sup>	4.21 <sup>a</sup>	4.20 <sup>a</sup>	0.093
Lactic acid / Mlečna kiselina	43.3 <sup>b</sup>	66.8 <sup>a</sup>	52.6 <sup>b</sup>	51.9 <sup>b</sup>	51.6 <sup>b</sup>	50.3 <sup>b</sup>	64.3 <sup>a</sup>	65.9 <sup>a</sup>	67.3 <sup>a</sup>	62.9 <sup>a</sup>	0.91
Acetic acid / Sirćetna kiselina	34.8 <sup>a</sup>	26.1 <sup>ab</sup>	27.3 <sup>ab</sup>	26.5 <sup>ab</sup>	26.3 <sup>ab</sup>	25.1 <sup>ab</sup>	23.3 <sup>ab</sup>	23.0 <sup>ab</sup>	22.4 <sup>b</sup>	22.6 <sup>ab</sup>	0.84
Propionic acid / Propionska kiselina	1.2 <sup>a</sup>	0.5 <sup>b</sup>	0.3 <sup>bc</sup>	0.3 <sup>bc</sup>	0.4 <sup>bc</sup>	0.2 <sup>c</sup>	0.0	0.0	0.0	0.0	0.05
Butyric acid / Buterna kiselina	8.8 <sup>a</sup>	2.6 <sup>b</sup>	1.2 <sup>b</sup>	1.4 <sup>b</sup>	0.9 <sup>b</sup>	0.9 <sup>b</sup>	0.0	0.0	0.0	0.0	0.03
Lactic acid % of total acid / Mlečna kiselina u % ukupnih kiselina	49.15 <sup>c</sup>	69.58 <sup>b</sup>	64.62 <sup>b</sup>	64.79 <sup>b</sup>	65.15 <sup>b</sup>	65.75 <sup>a</sup>	73.40 <sup>a</sup>	74.13 <sup>a</sup>	75.03 <sup>a</sup>	73.56 <sup>a</sup>	1.263

<sup>1, 2, 3</sup> Designations as in Table. 1. / Oznake kao u tabeli 1.

SEM – Standard error of means / Standardna greška srednje vrednosti

WA – Without additive / Bez aditiva

BE – With lactic acid bacteria and enzyme inoculant additive / Sa dodatkom bakterija mlečne kiseline i enzimskih inokulanata

B1, B2, B3, B4 – With rolled barley additive (50, 100, 150, 200  $\text{g}\cdot\text{kg}^{-1}$  of alfalfa forage) /

Sa dodatkom gnječnog ječma (50, 100, 150, 200  $\text{g}\cdot\text{kg}^{-1}$  lucerke)

BE-B1, BE-B2, BE-B3, BE-B4 – With rolled barley (50, 100, 150, 200  $\text{g}\cdot\text{kg}^{-1}$  of alfalfa forage) and lactic acid bacteria and enzyme inoculant additive

/ Sa dodatkom gnječnog ječma (50, 100, 150, 200  $\text{g}\cdot\text{kg}^{-1}$  lucerke) i bakterijom mlečne kiseline i enzimskim inokulantom

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

Srednje vrednosti sa različitim slovima u istom redu se signifikantno razlikuju

The results of fermentation quality in alfalfa-rolled barley silages are in agreement with the findings of *Harrison et al.* (1994) who showed that increasing proportion of rolled barley (50, 100 and 150 g·kg<sup>-1</sup> of alfalfa forage, DM content of 160, 240 and 350 g·kg<sup>-1</sup>) was accompanied by a steady decrease in the content of acetic and propionic acids down to the lowest values for alfalfa silages with the dry matter content of 240 g·kg<sup>-1</sup>. In the studies of *Harrison et al.* (1994), the bacterial and enzyme inoculants did not increase lactic acid in the silages but significantly reduced acetic and propionic acids in alfalfa silages with a DM content of 160 and 240 g·kg<sup>-1</sup>. Improved fermentation quality due to reduced acetic and propionic acid fermentation was also observed in grass or alfalfa silages supplemented with rolled barley (*Jones 1988; Moseley and Ramanathan, 1989; Brzóška et al., 2000*).

The content of nitrogen fractions in alfalfa and alfalfa-rolled barley silages made with or without the addition of bacterial and enzyme inoculants is shown in Table 3. The total-N content of silages without additives (WA) was 32.1 g·kg<sup>-1</sup>DM. The supplement of bacterial and enzyme inoculants to the ensiled alfalfa reduced the losses of total-N (34.8 g·kg<sup>-1</sup>DM) in BE silages. Ensiling alfalfa with rolled barley or with rolled barley and bacterial and enzyme inoculants did not cause significant changes in the total-N content of these silages compared to the total-N content of BE silages. A tendency was observed, however, towards decreased total-N content of silages with the increasing proportion of rolled barley in the mixtures. The total-N content was 35.8 and 37.6 g·kg<sup>-1</sup>DM in B1 and BE-B1 silages, and 33.9 and 34.7 g·kg<sup>-1</sup>DM in B4 and BE-B4 silages, respectively.

Alfalfa silages made without additives (WA) were characterized by the lowest content of protein-N (12.2 g·kg<sup>-1</sup>DM), indicating intensive proteolysis of protein in conditions favorable (pH – 5.31) to the activity of plant proteases. The process of protein proteolysis was observed to be greatly inhibited in alfalfa silages supplemented with the bacterial and enzyme inoculants (BE) in which the content of protein-N was 23.4 g·kg<sup>-1</sup>DM. Ensiling alfalfa-rolled barley mixtures with or without the addition of lactic acid bacteria and enzyme inoculants did not significantly affect protein proteolysis in the silages compared to BE silages. The content of protein-N ranged from 20.9 to 25.3 g·kg<sup>-1</sup>DM in B1 – B4 silages and from 23.6 to 25.3 g·kg<sup>-1</sup>DM in BE-B1 – BE-B4 silages.

The supplement of the bacterial and enzyme inoculants alone to the ensiled alfalfa forage significantly ( $P > 0.01$ ) inhibited the deamination process in BE silages in which the content of ammonia-N was 2.6 g·kg<sup>-1</sup>DM. In silages without additives (WA), the ammonia-N content was 5.4 g·kg<sup>-1</sup>DM. The supplement of rolled barley alone to the ensiled alfalfa did not significantly change the content of ammonia-N (2.7–2.9 g·kg<sup>-1</sup>DM) in B1 – B4 silages. Significant ( $P < 0.01$ ) limitation of the deamination process was found in alfalfa-rolled barley silages made with bacterial and enzyme inoculants (BE-B1 – BE-B4) in which the ammonia-N content was 1.8 – 1.9 g·kg<sup>-1</sup>DM.

In the present experiment, the highest ammonia-N content was in WA silages whose pH was 5.31. In the other silages, the ammonia-N content decreased with decreasing pH value. These findings indicate that pH is an important limiting factor of the deamination process due to clostridial bacteria whose optimum activity takes place in pH environments above 5.0 (*McDonald et al., 1991; Makoni et al., 1997*).

Table 3. Nitrogen fractions ( $\text{g}\cdot\text{kg}^{-1}\text{DM}$ ) in alfalfa and alfalfa-rolled barley silages made with or without addition of a lactic acid bacteria and enzyme inoculant\*

Tabela 3. Azotne frakcije ( $\text{g}\cdot\text{kg}^{-1}\text{SM}$ ) u silaži lucerke i silaži lucerke i gnječnog ječma sa ili bez dodavanja bakterije mlečne kiseline i enzimskog inokulanta\*

Item/ Stavka	Type of silage / Vrsta silaže										SEM
	WA	BE	B1	B2	B3	B4	BE-B1	BE-B1	BE-B3	BE-B4	
Total-N / Ukupni N	32.1 <sup>b</sup>	34.8 <sup>ab</sup>	35.8 <sup>a</sup>	35.5 <sup>a</sup>	35.2 <sup>ab</sup>	33.9 <sup>b</sup>	37.6 <sup>a</sup>	36.8 <sup>a</sup>	35.7 <sup>a</sup>	34.7 <sup>ab</sup>	0.56
Protein-N / Protein-N	12.2 <sup>c</sup>	23.4 <sup>ab</sup>	20.2 <sup>b</sup>	20.3 <sup>b</sup>	21.9 <sup>ab</sup>	21.3 <sup>ab</sup>	25.3 <sup>a</sup>	25.1 <sup>ab</sup>	24.8 <sup>ab</sup>	23.6 <sup>ab</sup>	0.87
NPN-N	19.9 <sup>a</sup>	11.4 <sup>bc</sup>	15.6 <sup>b</sup>	15.2 <sup>b</sup>	13.3 <sup>bc</sup>	12.6 <sup>bc</sup>	12.3 <sup>bc</sup>	11.7 <sup>bc</sup>	10.9 <sup>c</sup>	11.1 <sup>c</sup>	0.19
NH <sub>3</sub> -N	5.4 <sup>a</sup>	2.6 <sup>b</sup>	2.7 <sup>b</sup>	2.7 <sup>b</sup>	2.9 <sup>b</sup>	2.8 <sup>b</sup>	1.8 <sup>c</sup>	1.7 <sup>c</sup>	1.9 <sup>c</sup>	1.9 <sup>c</sup>	0.10
Protein-N % of total-N / Protein-N u % ukupnog N	30.01 <sup>c</sup>	67.24 <sup>a</sup>	56.42 <sup>b</sup>	57.18 <sup>b</sup>	62.21 <sup>ab</sup>	62.83 <sup>ab</sup>	67.29 <sup>a</sup>	68.21 <sup>a</sup>	69.47 <sup>a</sup>	68.01 <sup>a</sup>	0.59
NH <sub>3</sub> -N % of total-N / NH <sub>3</sub> -N u % ukupnog N	16.82 <sup>a</sup>	7.47 <sup>b</sup>	7.54 <sup>b</sup>	7.61 <sup>b</sup>	8.23 <sup>b</sup>	8.25 <sup>b</sup>	4.79 <sup>c</sup>	4.62 <sup>c</sup>	5.32 <sup>c</sup>	5.47 <sup>c</sup>	0.33

\* Designations as in Table 2. / Oznake kao u tabeli 2

<sup>a, b, c</sup> Means with different letters in the same row differ significantly ( $P < 0.01$ ) / Srednje vrednosti sa različitim slovima u istom redu se signifikantno razlikuju

The effect of effluent absorbents on protein degradation during forage fermentation has not been well documented in the literature. Brzóska *et al.* (1998) reports that absorbent supplements in the form of ground barley and dried sugar beet pulp to grass forages did not significantly limit the process of protein degradation during fermentation although it decreased the ammonia-N content of silages. A significant reduction in the ammonia-N content of silages, increasing with an increase in the proportion of rolled barley in alfalfa-rolled barley mixtures before ensiling was also observed by *Harrison et al.* (1994). The present results confirmed the earlier reports of the above authors for reduced protein degradation in silages made with absorbent in the form of rolled barley.

The effect of bacterial and enzyme inoculants on protein degradation in the silages has been poorly researched. *Huhtanen et al.* (1985) found that in grass silages supplemented with enzyme preparations, the proportion of soluble-N in total-N increases without a concurrent increase in the proportion of ammonia-N in total-N. These authors explained that during hydrolysis of cellulose under the effect of cellulolytic enzymes nitrogenous components of cell walls are released with no accompanying proteolytic degradation of cell-wall proteins and amino acids. The beneficial effects of enzyme preparations on limiting the process of protein degradation during fermentation of ensiled grass or alfalfa silages were also observed by *Hristov (1993) and Selmer-Olsen et al. (1993)*.

In the present paper, alfalfa and alfalfa-rolled barley silages made with the addition of lactic acid bacteria and enzyme inoculants were characterized by a high proportion of protein-N in total-N (67.21 to 69.47%) and a low proportion of ammonia-N in total-N (5.47 to 7.47%) compared to alfalfa silages made without additives (WA) in which the content of the above determinants of protein degradation was 38.01 and 16.81%, respectively. These results point to the high efficiency of the bacterial and enzyme inoculants used in inhibiting proteolysis and deamination during fermentation of alfalfa forage ensiled with or without rolled barley.

### Conclusions

The addition of rolled barley to ensiled alfalfa forage increases the dry matter and WSC content while reducing the content of crude protein, NDF and ADF in mixtures before ensiling. In alfalfa silages supplemented with rolled barley, no significant changes in the contents of crude protein, WSC and acetic acid were found compared to alfalfa silages supplemented with lactic acid bacteria and enzyme inoculants. Alfalfa silages supplemented with rolled barley were found to have increased contents of NDF and ADF and decreased contents of lactic, propionic and butyric acids compared to alfalfa silages made with lactic acid bacteria and enzyme inoculants. Ensiling alfalfa-rolled barley mixtures with the addition of bacterial and enzyme inoculants significantly limited crude protein and WSC losses during fermentation, reduced NDF and ADF contents, increased lactic acid fermentation, and completely inhibited propionic and butyric acid fermentation in the silages. In the present experiment, the supplement of

rolled barley to the ensiled alfalfa forage was found not to limit the processes of proteolysis and deamination in the silages compared to alfalfa silages supplemented with bacterial and enzyme inoculants. Significant ( $P < 0.01$ ) inhibition of protein proteolysis and deamination was found in alfalfa-rolled barley silage made with the addition of bacterial and enzyme inoculants. It should be emphasized that the best fermentation quality and the lowest protein degradation during fermentation were characteristic of the silages made with rolled barley (100 and 150  $\text{g}\cdot\text{kg}^{-1}$  of alfalfa forage) and lactic acid bacteria and enzyme inoculants.

## UTICAJ BAKTERIJSKOG INOKULANTA MLEČNE KISELINE SA DODATKOM ENZIMA I GNJEČENOG JEČMA NA HEMIJSKI SASTAV I DEGRADACIJU PROTEINA U SILAŽI LUCERKE

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### Rezime

U ovom ogledu, silirana je stočna hrana (suva materija 216.3  $\text{g}\cdot\text{kg}^{-1}$ ) koja potiče od 1. otkosa lucerke (*Medicago sativa* L.) cv, Radius, košene u ranom stadijumu cvetanja. Lucerka je silirana bez inokulanata; sa bakterijama mlečne kiseline i enzimskim inokulantom; sa gnječenim ječmom; ili sa gnječenim ječmom i bakterijom mlečne kiseline i enzimskim inokulantom. Gnječeni ječam je dodavan u količinama od 50, 100, 150 200  $\text{g}\cdot\text{kg}^{-1}$  stočne hrane od lucerke. Bakterijski enzimski inokulant (Bactozym) koji je sadržavao sledeće kulture *Lacto bacillus casei*, *Enterococcus faecium* M74 i *Pediococcus* sp. (ukupno  $15 \times 10^9$  CFU u 1g preparat koji se lako pretvara u prah) u prahu i kompleks celulaza-hemicelulaza (25000 n cat. u 1ml) i glukoza-oksidaza (4000 n cat. u 1ml) u tečnom delu. Inokulant je korišćen u vodenom rastvoru u količini od 10mg bakterijske komponente i 0.2ml enzimske komponente /  $\text{kg}^{-1}$  lucerke. Dodavanje samo gnječenog ječma lucerkinom krmivu nije imalo značajnog efekta na poboljšanje procesa proteoloze i deaminacije u ovoj vrsti silaže, u poređenju sa silažama od lucerke koje su pravljene uz pomoć bakterijskih i enzimskih inokulanata. Signifikantno manje sirovog proteina kao i gubici WSC-a u procesu fermentacije, povećan intenzitet fermentacije mlečne kiseline, nedostatak fermentacije propionske i buterne kiseline, i znatno redukovani proces degradacije proteina su pronađeni u silaži od lucerke i gnječenog ječma uz dodatak bakterije mlečne kiseline i enzimskog inokulanta.

**Ključne reči:** lucerka, silaža, bakterija mlečne kiseline i enzimski inokulant, absorbent tečne faze, kvalitet fermentacije, degradacija proteina

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