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EFFECTS OF LACTIC ACID BACTERIA INOCULATION ON MOUNTAIN GRASS AND ALFALFA SILAGE FERMENTATION CHARACTERISTICS

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Abstract

The objective of this work was to evaluate the effects of six lactic acid bacterial strains originated from different natural habitats of fermented food and feedstuff on pasture grass and alfalfa laboratory scale silages. The prepared mini silos were inoculated with the following bacterial strains alone or in combination: *Lactobacillus brevis* LbB ($2x10^7$ cfu/mL), *Enterococcus faecalis* EF ($3.6x10^8$ cfu/mL), *Lactobacillus plantarum* subsp. *plantarum* LbP ($2.8x10^8$ cfu/mL), *Weissella paramesenteroides* WP ($3.6x10^7$ cfu/mL), *Leuconostoc lactis* LL ($3.5x10^8$ cfu/mL), *Lactobacillus paracasei* subsp. *tolerans* LbPT ($8.1x10^8$ cfu/mL). The inoculation of the grass and alfalfa laboratory silos improved the chemical composition and fermentation patterns of the silages. Significant difference was detected ($P < 0.05$) in crude protein content, lactic acid production and acid detergent fibre and neutral detergent fiber content in some of the treated samples compared to the control. The best performing treatment in the case of grass silage was the *Lactobacillus brevis* LbB inoculant, for alfalfa silage the most effective was the *Enterococcus faecalis* EF inoculant. Also considerably good results were achived with the inoculant *Lactobacillus paracasei* subsp. *tolerans* LbPT. Our results indicate that a bacterial consortium containing *Lactobacillus brevis* LbB, *Enterococcus faecalis* EF and *Lactobacillus paracasei* subsp. *tolerans* LbPT could be a strategy for silage inoculant with fermentation acceleration and prevention of nutritive value loss for the achievement of high quality silages.

Keywords: fermentation, lactic acid bacteria, silage, silage inoculant

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1. Introduction

In Ciuc Mountains grasslands and alfalfa represent the principal sources of feedstuff in form of conserved forage. From nutrition value point of view the hay is not suitable continuously during the year. Important forage producing preservation method is ensiling. Ensilage of different cops, agricultural by-products are based on biochemical and microbiological processes, including the conversion of water soluble carbohydrates to fermentation end products (Jalč et al., 2009; Rooke and Hatfield, 2003; Pahlow et al., 2003).

Bacterial inoculants can be used to promote the fermentation patterns and preserve the nutritive value of the crops without substantial nutritive loss. These additives contribute to the faster decrease in pH, increase of organic acids and dry-matter, NH_3-N content, and reduce the protein degradation (Filya and Sucu, 2010; Liu et al., 2014; Tian et al., 2014). The microbiological inoculants are safe, easy-to use and environmentally friendly (Zhang et al., 2009). These

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products have different types, they can stimulate fermentation, inhibit undesirable microorganisms, and can be supplemented with nutrients or absorbents (Mannetje, 2007).

Lactic acid bacterial (LAB) inoculants can comprise homofermentative LAB (such as *L. plantarum, Enterococcus faecium*) or heterofermentative LAB (such as *L. bucneri, L. brevis*) (Zhang et al., 2009). This two groups of LAB with different hexose metabolism have their role in the fermentation of the silage and in the end products development. As heterofermentative LAB produce higher amount of acetic acid with antifungal properties, they inhibit the epiphytic microbial populations and improve the aerobic stability of silages (Daniel et al., 2015; Schmidt et al., 2014; Wambacq et al., 2013). It was shown that application of different types of LAB resulted in different effects on the silage quality (Ni et al., 2015). The impact is related more to the strain and less than to the species (Ávila et al., 2014).

LAB strains with beneficial properties isolated from natural habitats of fermented products are less studied and their function in ensiling process needs to be discovered and applied. Therefore, the objective of this study was to determine the effect of LAB inoculation (single strains or in consortium), on the fermentation characteristics of grass and alfalfa laboratory scale silages.

2. Materials and methods

2.1. Laboratory scale silage preparation

The grass and alfalfa used for ensiling process were harvested from the Ciuc Mountain in June 2014. The silage materials were chopped to a length of cut of 20-40 mm with a branch crusher. 100 g of fresh grass and alfalfa were ensiled in anaerobic polyethylene bags (200 mm x 280 mm, LAICA1 vacuum package), which were sealed with Laica Vacuum Sealer Machine. Before sealing, the mini bags were inoculated with the following LAB strains isolated by us: *Lactobacillus brevis* LbB ($2x10^7$ cfu/mL, isolated from sauerkraut juice), *Enterococcus faecalis* EF ($3.6x10^8$ cfu/mL, isolated from whey), *Lactobacillus plantarum* subsp. *plantarum* LbP ($2.8x10^8$ cfu/mL, isolated from cheese), *Weissella paramesenteroides* WP ($3.6x10^7$ cfu/mL, isolated from silage), *Leuconostoc lactis* LL ($3.5x10^8$ cfu/mL, isolated from goat cheese), *Lactobacillus paracasei* subsp. *tolerans* LbPT ($8.1x10^8$ cfu/mL, isolated from cheese). Population density of the inoculant stain was 10^7-10^8 cfu/mL. The control samples were made without inoculants. Before the inoculation, some alfalfa was supplemented with 15 g/kg sucrose. The lactic acid bacterial culture was applied at 1 mL per 100 g grass. These laboratory silos were kept at room temperature for 60 days. The mini silos were sampled for chemical analysis after 3, 8, 17, 30, 60 days and for microbiological analysis after 3 and 30 days. Silage samples were stored at -20 °C for further analysis (Cao et al., 2013; Hoedtke and Zeyner, 2011; Heinritz et al., 2012; Jalč et al., 2009).

2.2. Chemical analysis

The determination of lactic acid and volatile fatty acids including acetate, butyrate and propionate was performed by high performance liquid chromatography (HPLC), using Varian Pro Star 210, equipped with Transgenomic Coregel 87H3 column (Transgenomic, Inc., Omaha, USA) and a UV detector at 50 ºC. As mobile phase 0.8 mM H_2SO_4 was used, at a flow rate of 0.6 $mL.min^{-1}$.

Samples were mixed with distilled water in 1 to 9 ratios and extracted 30 seconds under vigorous mixing with an electrical blender. One mL of each filtrate was centrifuged for 5 minute at 14000 rpm, and injected in the HPLC (Sucu et al., 2011).

Crude protein content (CP) of the grass silages was determined by a Kjeldahl method with Velp Scientifica auto analyser. Dried samples were digested with sulphuric acid, potassium sulphate and selenium for 60 min at 420 ºC. The NH_3 was treated with NaOH. Neutral detergent fiber (NDF) and acid detergent fibre (ADF) were determined according to the method of Van Soest (Van Soest et al.,1991).The dried samples were treated with acid and neutral detergents, filtered and dried for 8 hours (Cao et al., 2013; Sadeghi et al., 2012).

Dry matter content (DM) of alfalfa and grass samples were determined by oven drying for 24 h at 67 ºC (Bakken et al., 2011).

2.3. Microbiological analysis

The presence of the undesirable microorganisms as *Clostridium* sp., *E. coli, Salmonella sp. Enterobacter aerogenes*, yeasts and moulds in the silage samples was determined. The presence of these microbes was detected with conventional microbiological methods on selective mediums: Brilliant Green Agar (lactose 10.0 g, sucrose 10.0 g, sodium chloride 5.g, pancreatic digest of casein 5g, peptic digest of animal tissue 5 g, yeast extract 3 g, phenol red 0.08 g, Brilliant Green 12.5 mg Agar 20.0 g), Tergitol-7 Agar (Oxoid), Clostridial Differential Broth (Merck) and Czapek Dox (Fluka) agar.

2.4. Statistical analysis

The means of results from treatments were analysed by one-way ANOVA using the Past3 free software. Differences between the treatment means were considered to be significant at 95% level ($P < 0.05$).

3. Results and discussion

For the production of well-preserved silage it is necessary to reduce the plant enzymes activities as proteolysis and to prevent the undesirable

microorganism's activity. Different moulds and bacteria cause the deterioration of silage (Zhang et al., 2009). In order to achieve these the number of LAB it has to be increased. These microbes can accelerate the pH reduction due to lactic acid production. Due to this process, the number and degradation activity of the yeast are decreased. Analysis of the chemical and microbiological characteristics of silage provide useful information for improving ensiling techniques.

In this study we tested the impact of using different LAB strains alone or in combination on the chemical composition and fermentation characteristics of grass and alfalfa silages during the fermentation. The applied inoculation in laboratory conditions on alfalfa silage rapidly reduced the pH value and increased the amount of lactic acid and acetic acid as well as inhibited the yeasts and moulds (data not shown).

The effect of inoculation with the six LAB strains, as single cultures and of two consortia on the chemical composition of grass silage is shown in Table 1. The inoculation of the grass silage with *L. brevis* LbB after 30 days of ensiling had significant positive effect on the CP (144.85 g/kg DM) compared to the control (P<0.05) (112.92 g/kg DM). The increase of the CP positively influenced the ensiling process. The protein degradation decreased during the fermentation.

The treatment with *L. brevis* LbB had no significant effect on ADF composition (P>0.05). The bacterial treatment did not affect the lignin and cellulose content of the samples compared to the control samples. Significant difference (P<0.05) can be observed in NDF composition and hemicellulose content. The NDF content (445.37 g/kg DM) and proportionally with this the amount of hemicellulose (95.21 g/kg DM) was reduced in the treated sample compared to the control (475.5 g/kg DM and 122.75 g/kg DM).

The inoculation with *E. faecalis* EF inhibited the protein degradation. A significant difference (P<0.05) in CP (146.06 g/kg DM) was detected in the treated sample compared to the control. The NDF (425.66 g/kg DM) and hemicellulose content (74.35g/kg DM) of the samples decreased compared to the control.

The *L. plantarum* LbP inoculation had significant effect on the crude protein content (150.72 g/kg DM) (P<0.05). This treatment prevented the protein degradation in maximum extent from the applied inoculations. The ADF (340.54 g/kg DM) and NDF (421.63 g/kg DM) content and hemicellulose content (81.09 g/kg DM) of the grass silage decreased significantly (P<0.05).

The inoculation with *L. paracasei* subsp. *tolerans* (LbPT) had no significant effect on CP and NDF content of the sample, however it had significant effect on ADF (378.17 g/kg DM) and hemicellulose content (P<0.05).

The treatment of the grass silage with *Ln. lactis* LL resulted in significant difference in the CP (P<0.05) (130.20 g/kg DM).This lactic acid bacterium inhibited the protein degradation during the fermentation. The application of *Ln. lactis* LL strain significantly reduced (P <0.05) the NDF (465.09 g/kg DM) and hemicellulose content (108.78 g/kg DM), but in lesser extent than the other LAB inoculations.

The application of *W. paramesenteroides* WP did not result in a significant effect on CP, but significantly influenced the ADF (373.81 g/kg DM) and NDF (482.59 g/kg DM) of the grass silage (P>0.05).

The treatment of the grass with a bacterial consortium consisting of *L. plantarum* LbP and *W. paramesenteroides* WP had no demonstrated significant effect on CP, but had significant effect on NDF(383.19 g/kg DM) and hemicellulose content(58.06 g/kg DM) (P<0.05) (Table 1).

Table 1. Effect of LAB inoculation on the chemical composition of grass silage

No.	***Sample***	***Crude protein content (g/kg DM)***	***Acid detergent fibre (g/kg DM)***	***Neutral detergent fiber (g/kg DM)***	***Hemicellulose (g/kg DM)***
1	Control	112.92 ± 7.90	352.8±2.60	475.5±1.67	122.75±0.96
2	LbB	144.85±5.90	350.16±4.88	445.37±2.19	95.21±5.57
3	EF	146.06±2.01	351.31±4.90	425.66±0.90	74.35±4.04
4	LbP	150.72±3.98	340.54±1.72	421.63±1.55	81.09±1.69
5	LbPT	126.98±2.32	378.17±2.70	481.01±6.53	102.84±8.28
6	LL	130.20±2.62	356.19±7.40	465.09±4.23	108.88±8.22
7	WP	119.96±4.28	373.81±2.56	482.59±1.41	108.78±3.60
8	LbP+WP	119.52±8.39	325.12±2.86	383.19±4.28	58.06±1.44
9	LbP+LbPT+LL	125.98±5.06	380.95±11.53	467.25±20.89	79.95±8.99
ANOVA	1vs2	*	ns	*	*
	1vs3	*	ns	*	*
	1vs4	*	*	*	*
	1vs5	ns	*	ns	*
	1vs6	*	ns	*	*
	1vs7	ns	*	*	*
	1vs8	ns	*	*	*
	1vs9	ns	*	*	*

*P < 0.05, with P-values of < 0.001 marked as "***", <0.01 marked as "**" and <0.05 marked as "*". Non significance was indicated as "ns".*

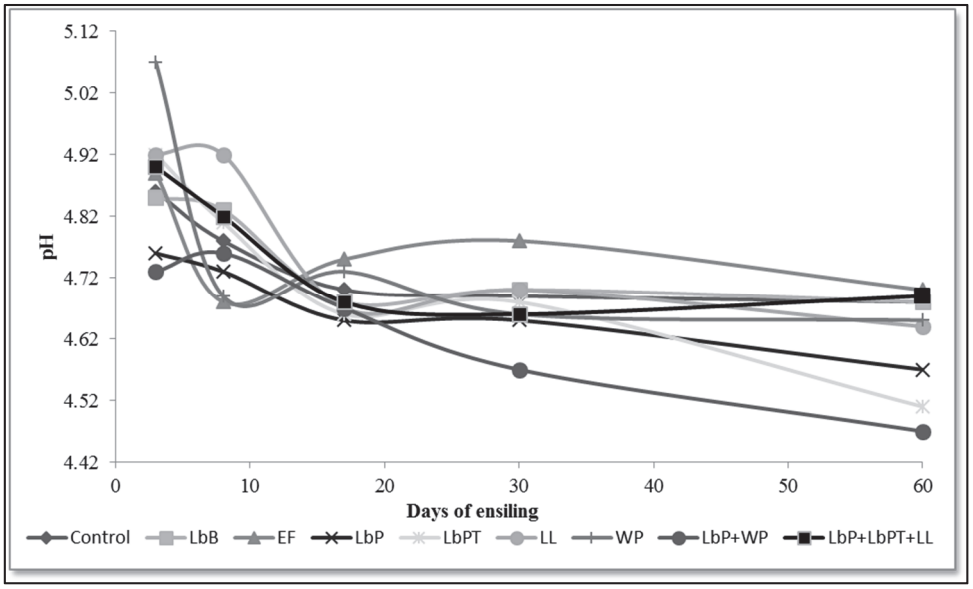

Fig. 1. pH levels of grass silages (with different LAB - and without inoculation)

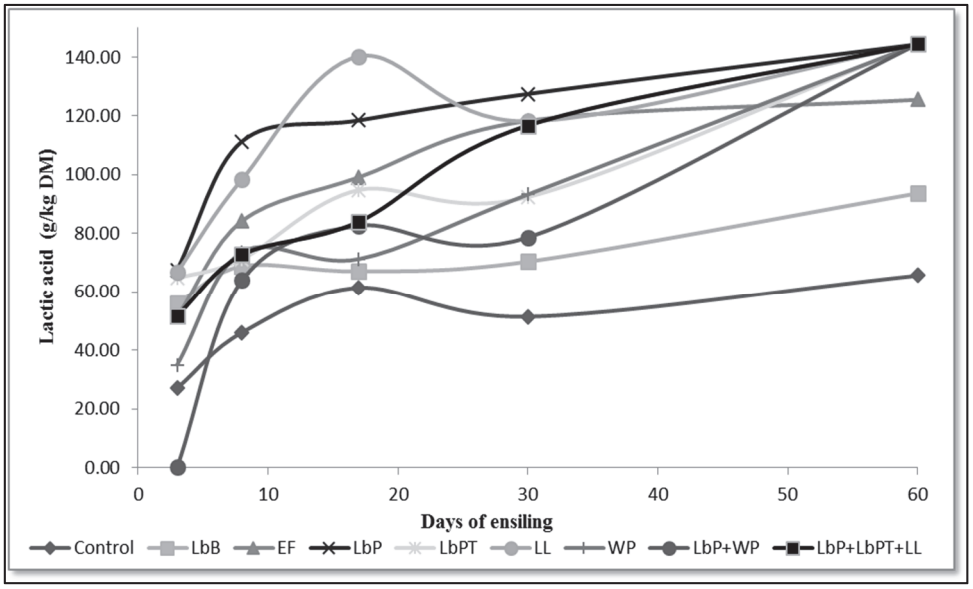

Fig. 2. Lactic acid content of grass silages (with different LAB - and without inoculation)

The treatment with the bacterial consortium of the three LAB *L. plantarum* LbP, *L. paracasei* subsp. *tolerans* LbPT and *Ln. lactis* LL resulted in significantly higher ADF content (380.95 g/kg DM) whereas the NDF (467.25 g/kg DM) and hemicellulose content (79.95 g/kg DM) decreased significantly compared to the control sample. The NDF content decrease indicates that part of the fibre, mainly hemicellulose, was solubilized. The determined values of CP of the inoculated samples in the present study were lower than the values of grass silage mown at first two stages found by De Boever et al. (2015). The differences observed can possibly be ascribed to different factors such as plant species or spacing, harvested stage of plant, consortia of the applied inoculant.

The effect of the different inoculation on fermentation characteristics of grass silage during the fermentation is shown in Fig. 1-4. In grass silages there were no big differences in the pH values of the treated and control samples (Fig. 1). The pH remained stable, as detected by Carvalho et al. (2015) or could be detected small differences, even though the acid concentration increased. This fact can be associated with the buffering capacity of grass forages. The inoculation with the following LAB: *L. brevis* LbB, *L. plantarum* LbP, *L. paracasei* subsp. *tolerans* LbPT and *Ln lactis* LL had significant increase of the lactic acid content as compared with the control sample (Fig. 2). In the case of *L. brevis* LbB the lactic acid concentration increased from 56.02 g/kg DM (third day) to 93.67 g/kg DM after up to 60 days of fermentation.

The highest concentration increase was detected in grass silage sample treated with the facultative heterofermentative *L. plantarum* LbP

LAB. This fact was observed by Seppälä et al. (2016) also in grass silage. The silages inoculated with *L. paracasei* subsp. *tolerans* LbPT showed an increase from 64.67 to 123.64 g/kg DM, whereas in the case of *Ln. lactis* LL the lactic acid concentration varied between 66.84-101.30 g/kg DM during the fermentation.

According to Santos et al. (2016) the decrease of the lactic acid concentration parallel with the increase of the propionic acid concentration is the result of *Clostridium propionicum* metabolism. This fact also was detected by us, in the silage samples treated with LAB consortia.

Inoculation with *L. brevis* LbB and *W. paramesenteroides* WP had a significant effect on acetic acid content as compared with the control sample (Fig. 3). The acetic acid content increased from 9.45 g/kg DM (third day) to 18.91 g/kg DM after up to 60 days of fermentation in the first mentioned case (Fig.3.). The high amount of acetic acid produced by heterofermentative LAB strains, as *Lactobacillus brevis* LbB, was detected by other researchers, too (Seppälä et al., 2016). This fermentation product contributes to the aerobic stability of the silages and inhibition of fungi (Zhang et al., 2009, Liu et al., 2014). In the samples inoculated with *W. paramesenteroides* WP we observed a high acetic acid content in the first stage of the fermentation that is reduced at the end of the fermentation (values varied between 14.98 -11.91 g/kg DM).

Inoculation with *L. brevis* LbB, *Ln lactis* LL, *W. paramesenteroides* WP and *L. plantarum* LbP strains resulted in a significant effect ($P<0.01$) on propionic acid content compared to control (Fig. 4).

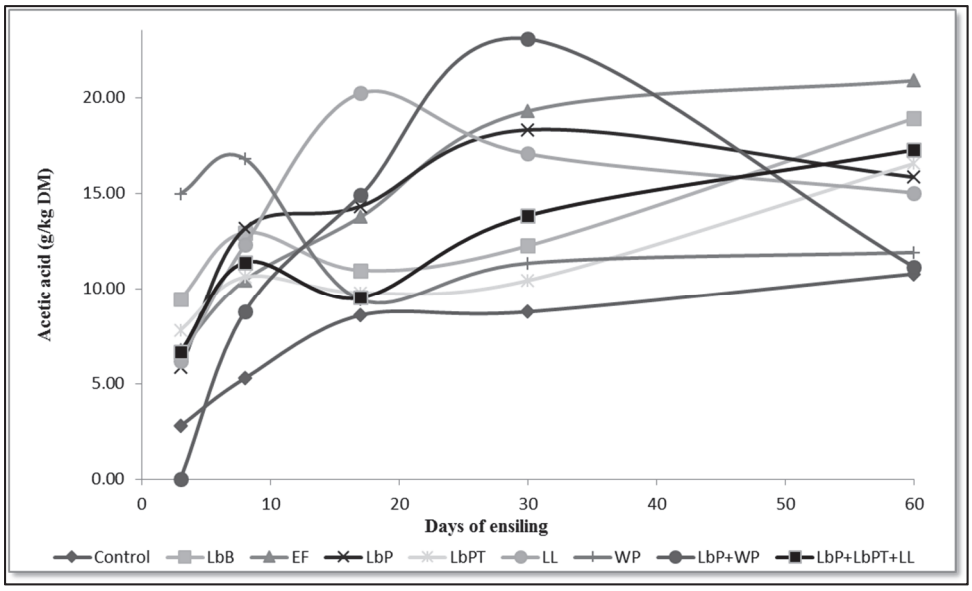

Fig. 3. Acetic acid content of grass silages (with different LAB - and without inoculation)

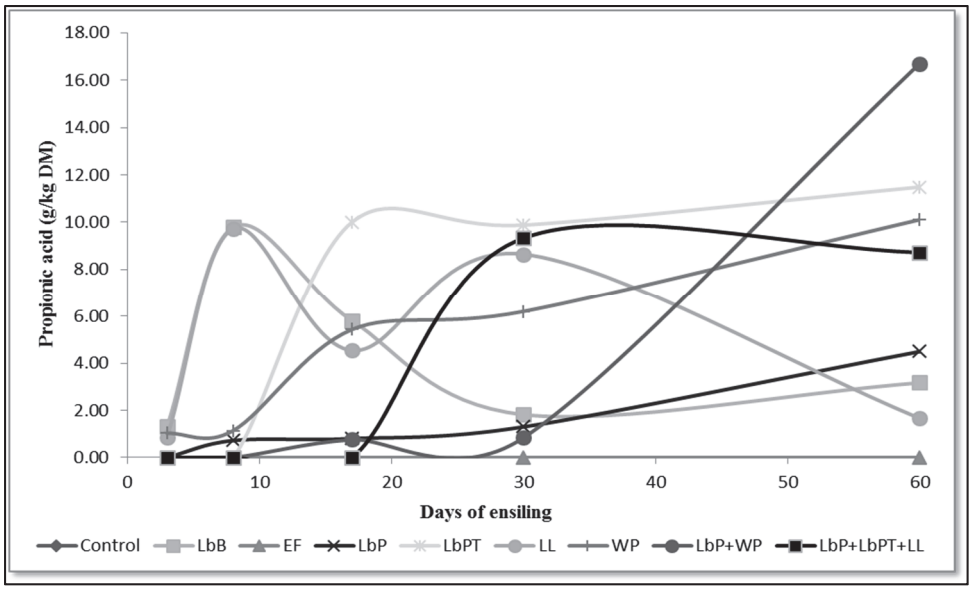

Fig. 4. Propionic acid content of grass silages (with different LAB - and without inoculation)

Table 2. Effect of the inoculation on chemical composition of alfalfa silage

No	*Sample*	*Crude protein content (g/kg DM)*	*Acid detergent fibre (g/kg DM)*	*Neutral detergent fiber (g/kg DM)*	*Hemicellulose (g/kg DM)*
1	Control	148.63±7.71	422.2±3.11	500±3.49	77.80±0.75
2	LbB	184.34±8.65	398.5±3.29	416.6±3.32	18.08±0.59
3	EF	196.31±5.25	331.8±30.4	356.4±1.83	24.54±2.75
4	LbP	185.24±4.36	342.9±3.83	400.9±1.74	58.04±5.05
5	WP+suc.	148.10±3.79	396.5±1.8	449.9±4.17	53.35±5.97
6	LL+suc.	137.68±2.70	371.7±3.62	428.2±3.01	56.47±5.83
7	LbPT+suc.	141.96±6.25	366.7±3.8	464.3±1.82	97.60±5.41
8	LbP+LbPT+suc.	118.41±1.86	410.7±3.33	475±2.09	63.73±3.96
9	LbP+WP+suc.	148.97±7.23	413.1±1.74	486±15.9	72.95±14.8
10	LbP+LbPT+LL+suc.	137.93±7.50	359.2±3.38	393.7±4.03	36.39±3.83
ANOVA	1vs2	*	*	*	*
	1vs3	*	*	*	*
	1vs4	*	*	*	*
	1vs5	ns	*	*	*
	1vs6	*	*	*	*
	1vs7	ns	*	*	*
	1vs8	*	*	*	*
	1vs9	ns	*	ns	ns
	1vs10	ns	*	*	*

*P < 0.05, with P-values of < 0.001 marked as "***", <0.01 marked as "**" and <0.05 marked as "*". Non significance was indicated as "ns".*

The effects of the inoculation on chemical composition of alfalfa silage are shown in table 2. The inoculation of the alfalfa silage with *L. brevis* LbB after 30 days of ensiling had a significant positive effect on the CP (184.34 g/kg DM) compared to the control (148.63 g/kg DM) (P<0.05). This inoculation positively influenced the ensiling process. The protein degradation decreased during the fermentation process. This finding can be associated with the high concentration of the lactic acid, detected also by other research (Tian et al., 2014). The treatment with *L. brevis* LbB also had a significant effect on ADF (398.5 g/kg DM), NDF (416.6 g/kg DM) composition and hemicellulose content (P<0.05). It was observed that heterofermentative inoculation of alfalfa silage resulted in higher DM losses during ensiling (Wambacq et al., 2013).

The inoculation with *E. faecalis* EF inhibited the protein degradation (P<0.05) (CP 196.31 g/kg DM), in greater degree than other treatments. This inoculation had significant effect on ADF (331.8 g/kg DM), NDF (356.4 g/kg DM) and hemicellulose content (P<0.05). The *L. plantarum* LbP inoculation had significant effect on CP (185.2 g/kg DM) (P<0.05), as previously observed by Zielińska et al. (2015) in the case of alfalfa silage.

As a result of this treatment, ADF (342.9 g/kg DM), NDF (400.9 g/kg DM) and hemicellulose content of the alfalfa silage significantly decreased comparing with the control (ADF 422.2 g/kg DM, NDF 500 g/kg DM). Inoculation with *W. paramesenteroides* WP of the sucrose-containing samples had no significant effect on the CP, being similar with the control sample (148.10 g/kg DM). This treatment resulted in significant decreases in ADF (396.5 g/kg DM), NDF (449.9 g/kg DM) and hemicellulose content (P<0.05), comparing with the control. Inoculation with *Ln. lactis* LL of the sucrose-containing samples had a significant effect on alfalfa silage CP (137.68 g/kg DM) (P<0.05), inhibiting the protein degradation as compared to the control. In this samples the ADF, NDF and hemicellulose content also decreased significantly (P<0.05). Inoculation with *L. paracasei* subsp. *tolerans* LbPT of the sucrose-containing samples had no significant effect on CP (P>0.05), but had significant effect on ADF (366.7 g/kg DM) content (P<0.05). The NDF content decreased significantly (464.3 g/kg DM) (P<0.05), whereas the treated sample had higher hemicellulose (97.60 g/kg DM) content compared to the control (P<0.05).

Inoculation with a mixture of *L. plantarum* LbP and *L. paracasei* subsp. *tolerans* LbPT of the sucrose-containing samples increased crude protein degradation of alfalfa silage (P<0.05). As the result of the treatment, the ADF, NDF and hemicellulose content was significantly reduced (P <0.05) compared to the control sample.

Proportionally increase of the fibre concentration can be associated with the DM loss of the silage resulted from the metabolism of the microorganisms during the fermentation (Santos et al, 2016). The CP of the alfalfa silages treated with a single strain did not differ from the values observed by Silva et al. (2016). The sucrose applied as additive with different LAB consortia did not showed a significant impact on the chemical composition of the silages. This result was unexpected because in previous research the addition of sucrose contributed to the improvement of silage characteristics (Zhang et al., 2015). Inoculation with a mixture of *L. plantarum* LbP, *W. paramesenteroides* WP of the sucrose-containing samples had a significant effect on ADF (413.1 g/kg DM) content (P<0.05).

The treatment with the bacterial consortia of the three LAB, *L. plantarum* LbP, *L. paracasei* subsp.

tolerans LbPT and *Ln. lactis* LL of the sucrose-containing samples resulted in a significant difference of the CP of the samples (137.93 g/kg DM) (P>0.05). This treatment also decreased the ADF, NDF (359.2±3 g/kg DM, 393.7 g/kg DM) and hemicellulose content of the alfalfa silage (36.39 g/kg DM)(P<0.05).

The effect of the different inoculation on fermentation characteristics of alfalfa silage during the fermentation is shown in Figs. 5-8. The *L. plantarum* LbP inoculation of the sucrose containing samples on alfalfa silages resulted in a significant effect on pH values (P<0.05) (Fig. 5) compared to the control. The treated samples had higher lactic acid concentration than control sample. Significant differences (P<0.05) in lactic acid content were detected in samples treated with *E. faecalis* EF, *L. plantarum* LbP, and in sucrose containing samples inoculated with a single LAB strain (Fig. 6). The highest lactic acid concentration increase was detected in grass silages treated with *E. faecalis* EF. The lactic acid concentration increased from 160.75 g/kg DM (third day) to 225.16 g/kg DM after up to 60 days of fermentation and the acetic acid concentration from 24.59 g/kg DM to 32.66 g/kg DM (Fig.7.). In the case of *L. plantarum* LbP the lactic acid content was between 109.74 g/kg DM and 205.76 g/kg DM, the acetic acid content varied between 8.56-37.75 g/kg DM.

Inoculation with the homofermentative LAB *L. plantarum* in both alfalfa and grass silages increased the lactic acid concentration as detected by others too (Zhang et al., 2009). Alfalfa is one of the leguminous plants that are very difficult to ensile due to their low water soluble carbohydrate content and high buffering capacity. To improve forage ensiling, sucrose was applied as additive with different LAB consortia. This type of inoculation resulted in a significant effect on alfalfa silage sample, as showed by Zhang et al. (2015).

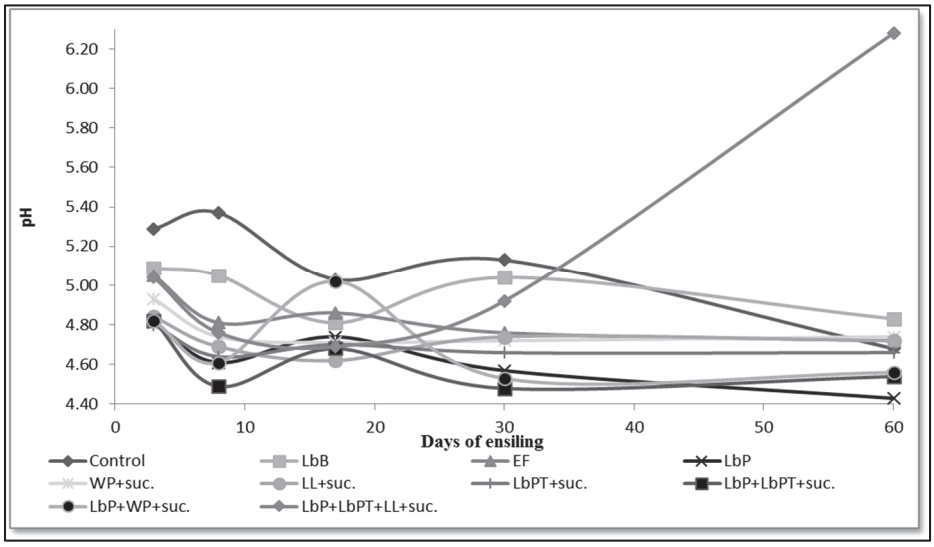

Fig. 5. pH levels of alfalfa silages (with different LAB - and without inoculation)

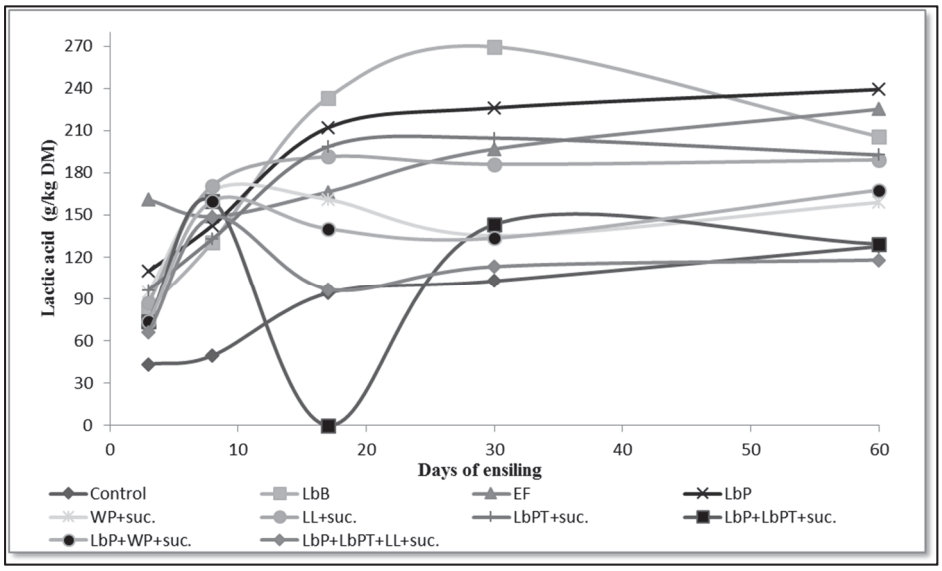

Fig. 6. Lactic acid content of alfalfa silages (with different LAB - and without inoculation)

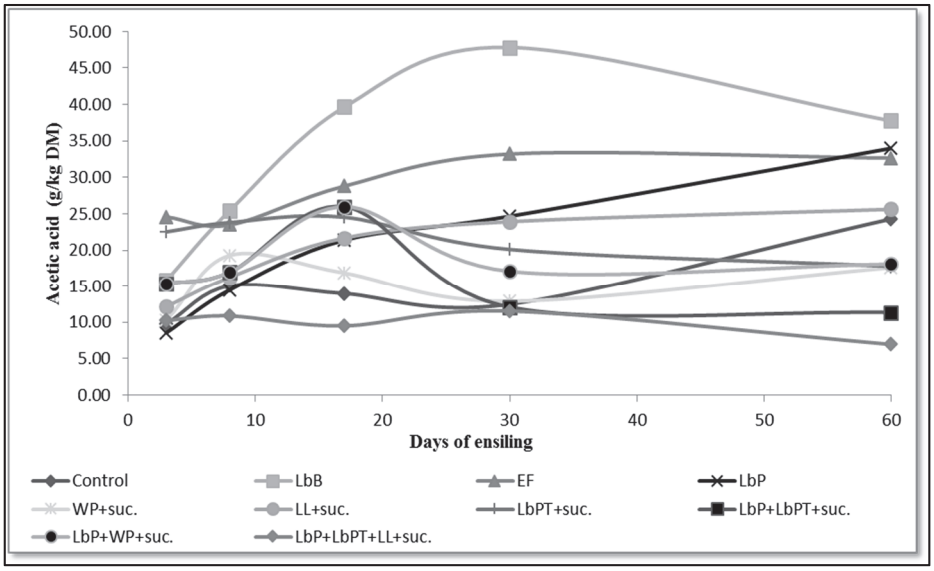

Fig. 7. Acetic acid content of alfalfa silages (with different LAB - and without inoculation)

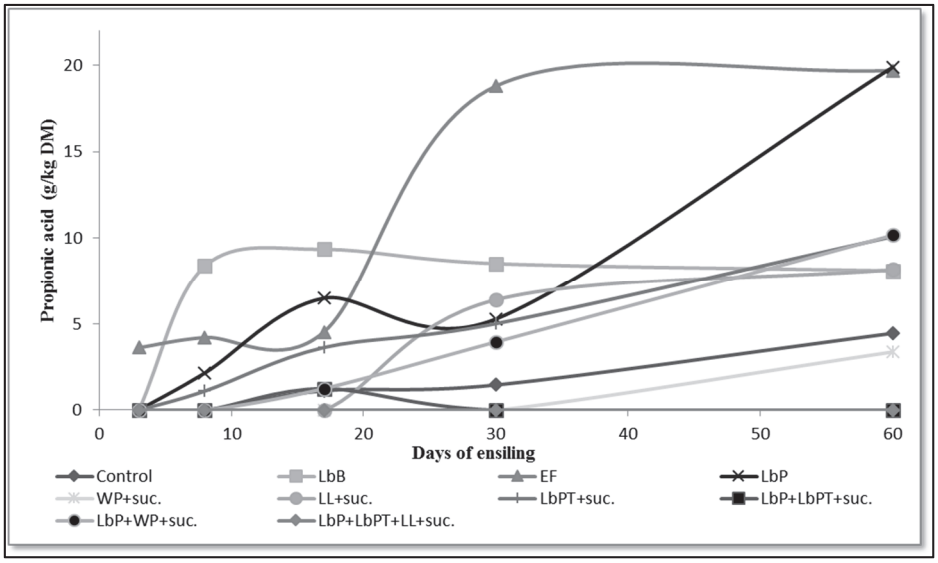

Fig. 8. Propionic acid content of alfalfa silages (with different LAB - and without inoculation)

Propionic acid content of the inoculated silage samples was different among treatments (Fig. 8.), observed also by Silva et al. (2016). In some cases the effect could not be detected. Except one sample, it was detected on the eighth day of fermentation. With the acceleration of the fermentation the butiric acid production is inhibited, as detected in the case of *E. faecium, L. fermentum, L. plantarum* (Jalč et al., 2009).

Based on the microbiological analysis, the presence of *E. coli* could be detected in raw grass, in the control and in the 3 days of *E. faecalis* EF treated sample, but in the 30 days-treated sample it was no longer present. *Clostridium* sp. was detected only in the beginning of the fermentation, but the LAB inoculation inhibited the activity of these bacteria. The butyric acid was not detected. *Enterobacter aerogenes* and *Salmonella* were not detected in the samples. In the case of alfalfa silage *E. coli* was not detected in any of the samples. *Enterobacter aerogenes* was detected only in fresh alfalfa samples.

The silage inoculants had positive impact on the protein content of the silage. Similarly with our results, the use of *L. buchneri* reduced the crude protein degradation of red clover and ryegrass compared with the untreated samples (Wambacq et al., 2013). The organic acid content of the treated silage samples with LAB originating from natural habitats is higher compared to the controls as shown by others (Jalč et al., 2009). Guo et al. (2014) reported that inoculation with *L. plantarum,* and *E. faecium* in *Pennisetum purpureum* Sch. silage inhibited the butiric acid formation. *L. plantarum* had improved silage quality compared to the control regardless of harvest time. In case of heterofermentative strains the fermentation profile is heterolactic and lower dry matter recovery can be detected, together with increased concentration of acetic acid, similarly with

the application of *L. buchneri* for maize silage (Keles et al., 2011).

The treatment with *L. plantarum* LbP of both silages in our study quickly resulted in high lactic acid formation. When this LAB strain was applied in consortium with other bacterial strains, the lactic acid production was lower. Presumably *L. plantarum* subsp. *plantarum* inhibited adjacent LAB and due to competitive inhibition in consortium with this strain, the treatment was not so efficient.

4. Conclusions

LAB applied as inoculants for silages had a benefic effect. Based on our results it was observed that the best performing treatment in the case of grass silage was the *L. brevis* LbB inoculant, while for alfalfa silage the most effective was the *E. faecalis* EF inoculant. Also the inoculant *L. paracasei* subsp. *tolerans* LbPT *can enhance the silage quality.*

A bacterial consortium containing three LAB strains, namely *L. brevis* LbB*, E. faecalis* EF *and L. paracasei* subsp*. tolerans* LbPT with good compatibility results, could be used as silage inoculant with fermentation acceleration and prevention of nutritive value loss for the achievement of high quality silages.

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