

THE EFFECTS OF INOCULANT LACTIC ACID BACTERIA ON THE FERMENTATION AND AEROBIC STABILITY OF SUNFLOWER SILAGE

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SUMMARY

This study was carried out to determine the effects of lactic acid bacterial inoculant on the fermentation and aerobic stability of sunflower silages. Sunflower was harvested at the milk stage. Inoculant-1174 (Pioneer®,USA) was used as homofermentative lactic acid bacterial inoculant. Inoculant was applied 6.00 log₁₀ cfu/g silage levels. Silages with no additive served as controls. After treatment, the chopped sunflower was ensiled in the PVC type laboratory silos. Three silos for each group were sampled for chemical and microbiological analysis on days 2, 4, 7, 14, 21, 28 and 56 after ensiling. At the end of the ensiling period, all silages were subjected to an aerobic stability test for 14 days. Neither inoculant improved the fermentation parameters of sunflower silages. At the end of the ensiling period, inoculant increased lactic acid bacteria (LAB) and decreased yeast and mould numbers of silages. Inoculant treatment did not affect aerobic stability of silages.

Key-words: homofermentative lactic acid bacterial inoculant, fermentation, aerobic stability, sunflower silage

INTRODUCTION

In recent years, the sowing of fodder crops during the rainy season (January to March) has become very popular. Generally, corn and sorghum are used, because they produce a well-preserved silage of good nutritive value. However, their dry matter (DM) yields and quality are uncertain from year to year, because of frequent drought stress.

Sunflower stands out as an alternative for forage production and conservation as silage because of its drought tolerance, high DM yields, resistance to cold and heat, adaptability to different edafoclimatic conditions and relative independence of latitude, altitude and photoperiod (Cotte 1959; Tomic, 1999). However, high fiber content of sunflower silage causes decreases in digestibility of nutrient matters (Demirel et.al., 2006; Ozduven et al, 2009). Sunflower can be used to ensile, but the ensiling and nutritional quality depend upon the stage of maturity at the harvest time (Tan and Tumer, 1996; Garcia, 2002; Toruk, 2003; Toruk et. al., 2009).

The application of silage additives has become the conventional implement to control the ensiling process. Although the main objective in using silage additives is to ensure the fermentation process to produce well preserved silages, attention is also paid to methods of reducing ensiling losses and improving aerobic stability of silages during the feed-out period (McDonald, 1991). In order to improve the ensiling process various chemical and biological additives have been developed. Biological additives are more suitable because they are safe and easy to use, non corrosive to machinery, do not pollute the environment, and are natural products (Sucu and Filya, 2006). Bacterial inoculants generally increase lactic acid and reduce pH, acetic acid, butyric acid and ammonia-nitrogen levels in silage (Sheperd et.al., 1995; Aksu et.al., 2004). Inoculation of forage crops with homofermentative lactic acid bacteria (LAB) can improve silage fermentation if sufficient fermentable substrate (WSC) is available.

The aim of this study was to determine the effects of homofermentative LAB inoculant on the fermentation and aerobic stability characteristics of sunflower silages

MATERIALS AND METHODS

Materials and silage preparation

Sunflower was harvested at the milk stage of maturity ($17.57 \pm 0.75\%$ DM). The whole plants were chopped about 3-5 cm and ensiled in PVC types silos with three replications. Three PVC silos from each group were sampled for chemical and microbiological analysis on days 2, 4, 7, 14, 28 and 56 after ensiling. At the end of the ensiling period, the silages were subjected to an aerobic stability test for 14 days .

The following treatments were used in the experiment:

Control: no additive

Inoculant: Inoculant-1174 (Pioneer®,USA) containing *Lactobacillus plantarum* and *Enterococcus faecium*. Final application rate of $6.00 \log_{10}$ colony forming units (cfu) LAB/g of fresh sunflower.

The application rate determined by the manufacturer stated the level of LAB in the products. On the day of the experiment, the inoculant was suspended in 600 ml of tap water and the whole suspension was sprayed over 360 kg (wet weight) of the chopped forage spread over a 1 x 4 m area. Inoculant was applied to the forages in a uniform manner with constant mixing.

Analytical procedures

Chemical analyses were performed in triplicate. The DM content of the fresh materials was determined by drying at 60°C for 48 h in a fan-assisted oven (Akyıldız, 1984). According to British standard method (Anonymous, 1986) pH was measured in fresh and material and silage samples. Buffering capacity (Bc) in fresh material was estimated as described by Playne and McDonald (1966). The ammonia nitrogen ($\text{NH}_3\text{-N}$) content of silages was determined, according to Anonymous (1986). The WSC content of silages was determined by spectro-photometer (Shimadzu UV-1201, Kyoto, Japan) after reaction with an antron reagent Anonymous (1986).

Crude protein (CP), and crude fiber (CF) were determined following the procedure of Association of Official Analytical Chemists (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF), as well as acid detergent lignin (ADL) were analysed using the sodium sulphite addition method without α -amylase and expressed with residual ash (Van Soest et al., 1991). Hemicellulose was calculated as the difference between NDF and ADF and cellulose as the difference between ADF and ADL.

Microbiological evaluation included enumeration of lactobacilli on pour-plate Rogosa agar (Oxoid CM627, Oxoid, Basingstoke, UK), and yeast and molds on spread-plate malt extract agar (Difco, Detroit, MI, USA) acidified with lactic acid to pH 4.0. Plates were incubated for 3 days at 30°C (Seale et. al., 1986). All microbiological data were transformed to \log_{10} .

The statistical analysis of the results included one-way analysis of variance and Duncan multiple range tests which were applied to the results using the Statistical Analysis System (1988).

Aerobic stability test

The silages stored for 56 days in the experiment were used. After emptying a PVC, half of the initial contents was again put into the bottle without compaction. The top was left uncovered, and a thermometer was placed in the centre of the silage. The PVC was kept in a room maintained at $20\text{-}23^{\circ}\text{C}$, whereas daily changes in the temperature were recorded for 14 days. Aerobic deterioration was considered to have started when the difference between the silage and surrounding air reached 2°C (Chen et al., 1994)

RESULTS

The chemical composition of the fresh and ensiled sunflower silage is given in Table 1. All silages were well preserved. In the experiment LAB inoculant did not improve the fermentation parameters of sunflower silages. The pH of all silages decreased faster and to a greater extent. During fermentation, significant difference was shown between the pH values of control and inoculated silages ($P < 0.01$; Figure 1). In the experiment the WSCs in all silages decreased with pH decrease. The inoculant treatments did not affect the concentration of WSC and $\text{NH}_3\text{-N}$ of the silages (Figure 2 and 3). After 4 days of ensiling, the inoculated silages had higher lactic acid and lower acetic acid levels compared to control silages ($P < 0.05$; Figure 4 and 5). The same trend was shown on 14th, 21st, 28th and 56th day of ensiling. During fermentation no butyric acid was present in the silages. The microbial composition of the sunflower silages is given in Table 1. LAB numbers increased ($P < 0.01$) and yeast numbers decreased in sunflower silages compared to the control silages

Table 2 presents the results of the aerobic exposure test of sunflower silages. Silage deterioration indicators are pH, temperature change and increase in yeast and mold numbers. The inoculated silages had lower pH, mould and yeasts numbers than the control silages.

Table 1. Chemical analysis of the sunflower silages

Tablica 1. Kemijska analiza silaža suncokreta

Item	At time ensiling	Control	Inoculant	P
pH	6.25	3.84±0.00	3.76±0.01	0.01
Bc, mEq NaOH/kg DM	39.17	-	-	
DM, % in FM	17.57	19.12±0.72	18.84±0.63	NS
$\text{NH}_3\text{-N}$, g/kg DM	-	1.21±0.17	0.98±0.10	NS
WSC, g/kg DM	86.82	23.80±0.83	24.00±0.81	NS
Lactic acid, % FM	-	1.51±0.06	2.10±0.17	0.05*
Acetic acid, % FM	-	1.76±0.48	0.65±0.18	0.05*
LAB, log ₁₀ cfu/g FM	2.57	3.90±0.02	5.12±0.01	0.01
Yeasts, log ₁₀ cfu/g FM	-	5.86±0.02	5.47±0.03	NS
Moulds, log ₁₀ cfu/g FM	-	NF	NF	
Crude protein, %DM	9.40	9.09±0.02	9.06±0.04	NS
NDF, % DM	44.41	45.71±2.71	44.31±0.72	NS
ADF, % DM	39.79	40.77±2.07	38.81±0.398	NS
ADL, % DM	12.61	11.67±1.33	9.11±2.16	NS
Hemicellulose, DM	4.62	4.94±0.70	5.49±1.12	NS
Cellulose, %DM	27.18	29.10±0.11	29.70±0.30	0.01**
EE, % DM	3.45	3.09±0.03	3.28±0.07	NS
Ash, %DM	9.12	9.37±0.11	9.16±0.12	NS

Bc: Buffering capacity, DM: Dry matter; $\text{NH}_3\text{-N}$: Ammonia nitrogen; WSC: Water soluble carbohydrate; LAB: lactic acid bacteria; NDF: Neutral detergent fiber; ADF: Acid detergent fiber ADL: Acid detergent lignin; Hemicellulose: NDF- ADF; Cellulose: ADF-ADL; EE: Ether extract; log cfu, logarithm colony forming unit; FM: Fresh Matter; NF: Not Found; NS: Not Significant. *and ** denote significance level of 0.05 and 0.01, respectively.

Table 2. Results of the aerobic stability test of the sunflower silages

Tablica 2. Rezultati testa aerobne stabilizacije silaža suncokreta

Items	Control	Inoculant	P
pH	8.78±0.06	8.28±0.51	NS
DM,% in FM	21.96±0.44	20.64±1.17	NS
WSC, g/kg KM	-	-	-
LAB, log ₁₀ cfu/g FM	NF	NF	-
Yeast, log ₁₀ cfu/g FM	6.82±0.01	6.29±0.02	NS
Mould, log ₁₀ cfu/g FM	3.28	3.21	NS

DM: Dry matter; FM: Fresh Matter; WSC: Water soluble carbohydrate; LAB: Lactic acid bacteria; NF: Not Found; NS: Not Significant

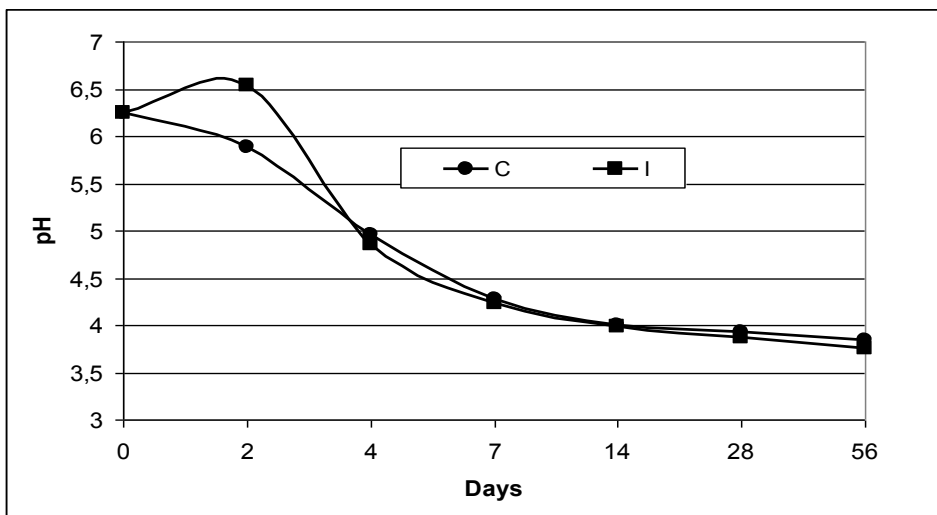


Figure 1. pH change in sunflower silages
Slika 1. Promjena pH u silažama suncokreta

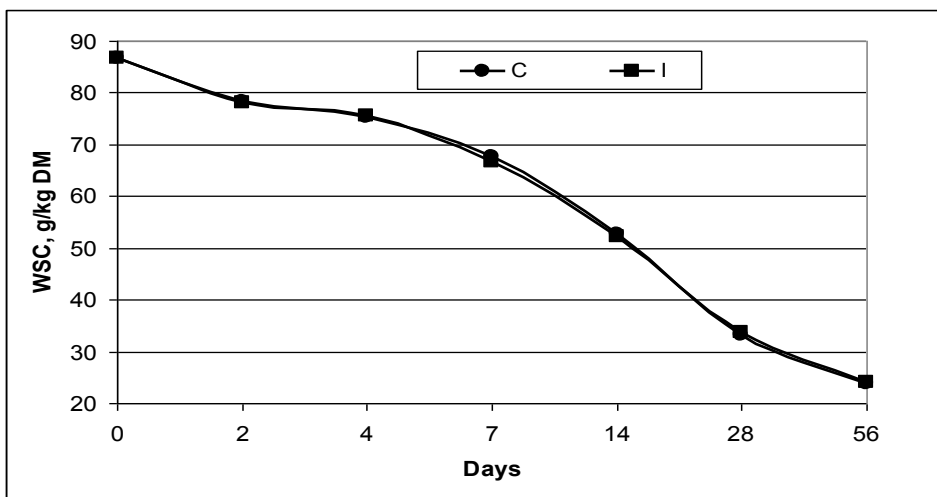


Figure 2. Water soluble carbohydrate (WSC) change in sunflower silages
Slika 2. Promjena vodo-topivog ugljikohidrata (WSC) u silažama suncokreta

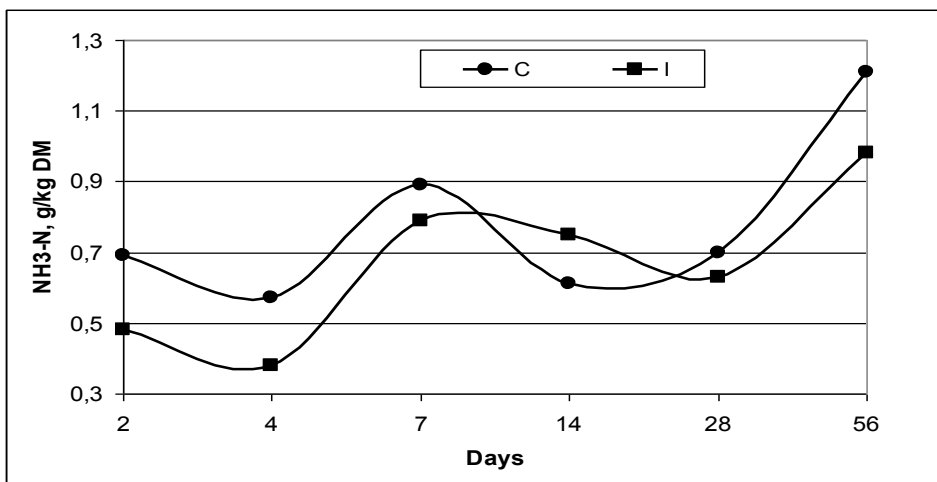


Figure 3. NH₃-N change in sunflower silages
Slika 3. Promjena NH₃-N u silažama suncokreta

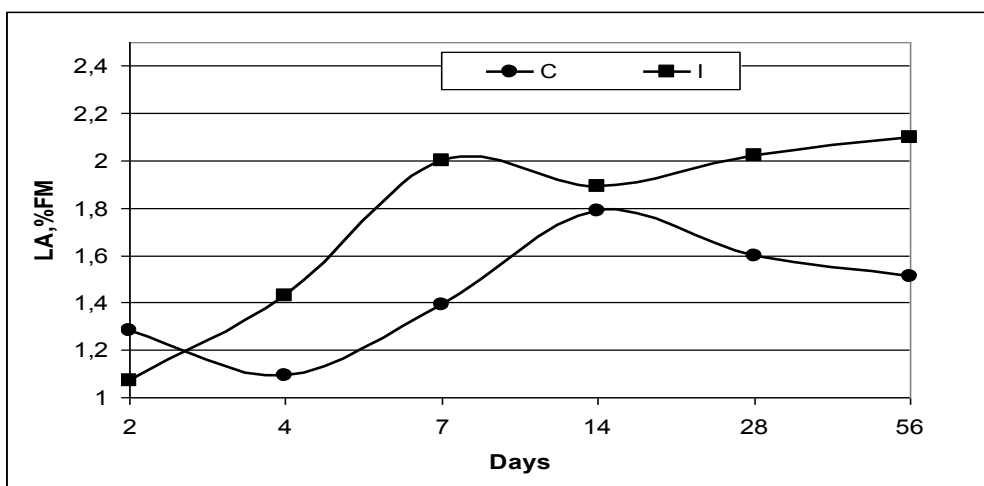


Figure 4. Lactic acid concentration change in sunflower silages
Slika 4. Promjena mliječno-kisele koncentracije u silažama suncokreta

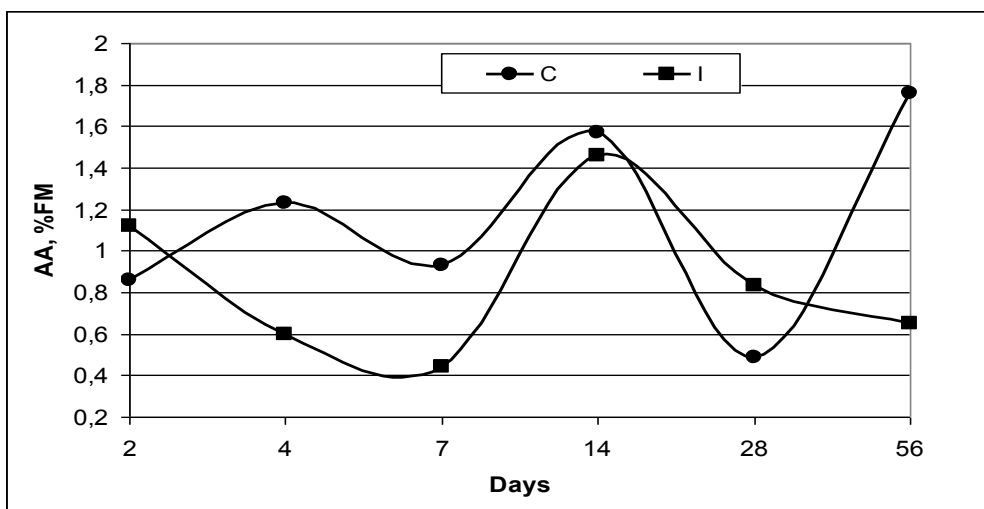


Figure 5. Acetic acid concentration change in sunflower silages
Slika 5. Promjena koncentracije octene kiseline u silažama suncokreta

DISCUSSION

The success of a bacterial inoculant as a silage additive depends on many factors, such as the type and properties of the crops to be ensiled, climatic conditions, epiphytic microflora, ensiling technique and the properties of the inoculant (Henderson, 1984). Until now, homofermentative LAB inoculants have been added to silage in order to stimulate lactic acid fermentation, accelerating the decrease in pH and thus improving silage preservation. In this experiment, homofermentative LAB inoculant did not improve lactic acid production sunflower silages. During fermentation inoculant increased lactic acid and decreased acetic acid production in silages. Bolsen et al. (1989) concluded that the whole crop corn was fermented rapidly and that bacterial inoculants had little effect on the rate and efficiency of silage fermentation. Observations reported by other researches (Buchanan et. al., 1981; Moon, 1981) were similar, and the present finding was confirmed by the earlier conclusions. Seale (1986), in his review on bacterial inoculants for silages, reported that suitable fast acid producing strains in sufficient numbers might be as effective as silage additives if the DM and WSCs of the crop are high enough. In the present study, all silages had lower pH values at an earlier stage of ensiling. LAB inoculants did

not affect concentrations of $\text{NH}_3\text{-N}$ of sunflower silages compared to control silage (except 2nd day) McDonald et al., (1991) reported that lower pH values inhibited protein degradation in silages. Therefore, concentrations of $\text{NH}_3\text{-N}$ of all sunflower silages were low in the experiment.

At the end of the ensiling period, LAB inoculants improved the microbiological composition of sunflower silages as expected. LAB inoculant increased LAB and decreased yeast and mould numbers of sunflower silages compared to the control silages. These findings are in agreement with those reported by Spoelstra (1991), Filya (2003), Sucu and Filya (2006) and Ozduven et al., (2009).

Table I shows fibre composition, CP, ether extract (EE) and ash content of the ensiled sunflower after 56th days. No differences were detected among treatments for CP, EE, ash, NDF, ADF, ADL and hemicellulose. Some differences were noted among treatments in CP, EE, ash, NDF, ADF, ADL and hemicellulose, but were most likely a consequence of sampling variation. However inoculant affected only cellulose contents ($P < 0.01$). LAB inoculant decreased cellulose content sunflower silages compared to the control silages.

Based on temperature changes, LAB inoculated silage was considered to have deteriorated after exposure to air (Figure 6). The silage temperature peaked after 4 days at 6°C above the ambient and cooled quickly thereafter. The control silage appeared resistant to aerobic deterioration.

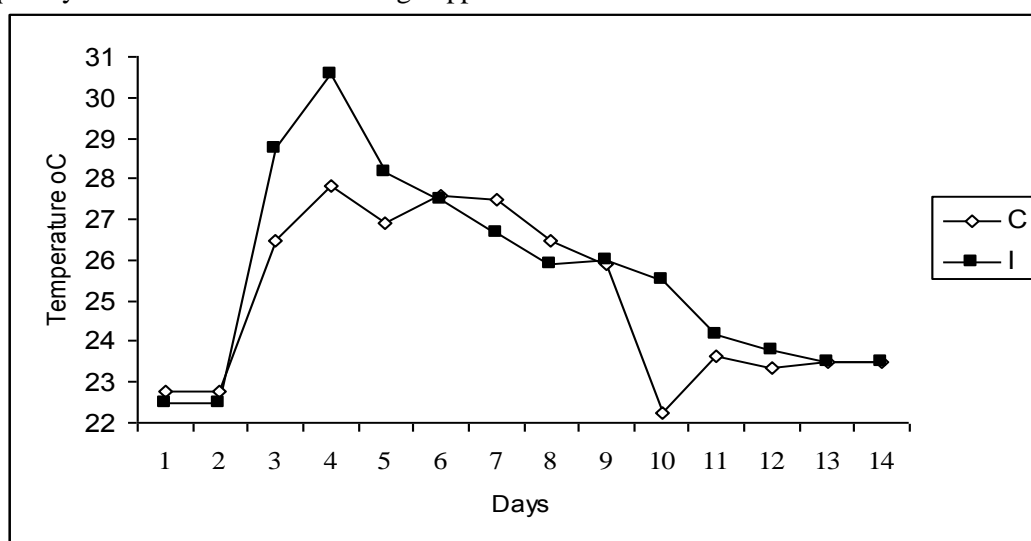


Figure 6. Changes in temperatures after aerobic stability exposure of sunflower silages

Slika 6. Promjene temperatura nakon ekspozicije aerobne stabilnosti silaža suncokreta

Filya *et al.* (2000) hypothesized that homofermentative LAB inoculants produced mainly lactic acid, which could serve as a substrate for lactate-assimilating yeasts upon aerobic exposure. Thus, only small amounts of shortchain volatile fatty acids (VFAs) such as acetic, propionic and butyric acids are produced. These VFAs can inhibit yeasts and molds, making silages treated with homofermentative LAB inoculants deteriorate faster upon exposure to air. This difference between our results and those published by Ohyama *et al.* (1975) and Pahlow (1982) is probably due to the fact that these researchers infiltrated air into the silage during the ensiling period from the beginning.

CONCLUSION

The results of the present study showed that homofermentative LAB inoculant did not improve the fermentation parameters or aerobic stability of sunflower silage.

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UTJECAJ INOKULATA MLIJEČNO KISELIH BAKTERIJA NA FERMENTACIJU I AEROBNU STABILNOST SILAŽE SUNCOKRETA

SAŽETAK

Ovo istraživanje imalo je za cilj odrediti utjecaj inokulata mliječno kiselih bakterija na fermentaciju i aerobnu stabilnost silaža suncokreta. Žetva suncokreta obavljena je u mliječnoj zriobi. Inokulat-1174 (PioneerR, USA) koristio se kao homofermentativni inokulat mliječno kiselih bakterija. Inokulat primijenjen je na razinama 6.00 log 10 cfu/g. Kontrole su bile silaže bez aditiva. Nakon tretiranja, sjeckani je suncokret siliran u PVC laboratorijskim silosima. Uzorci su uzimani iz 3 silosa za svaku grupu te kemijski i mikrobiološki analizirani 2., 4., 7., 14., 21., 28. i 56. dana nakon siliranja. Na kraju siliranja sve su silaže testirane na aerobnu stabilnost u periodu od 14 dana. Inokulat nije poboljšao fermentacijske parametre silaža suncokreta. Na kraju procesa siliranja inokulat je povećao mliječno kisele bakterije (LAB), a smanjio kvasac i plijesan u silažama. Postupak inokulatom nije utjecao na aerobnu stabilnost silaža.

Ključne riječi: homofermentativni inokulat mliječno kiselih bakterija, fermentacija, aerobna stabilnost, silaža suncokreta

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