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EFFECTS OF *LACTOBACILLUS PLANTARUM* INOCULANTS ON MAIZE SILAGE QUALITY

Snežana Đorđević[1], Violeta Mandić[2], Dragana Stanojević[3], Nataša Jovanović Ljesković[4]

[1]Faculty of Agriculture, University of Belgrade, Belgrade, Republic of Serbia
[2]Institute for Animal Husbandry Belgrade-Zemun, 11080, Zemun, Republic of Serbia
[3]Biounik d.o.o., Šimanovci, Republic of Serbia
[4]Faculty of Pharmacy, University Business Academy in Novi Sad
Corresponding author: violeta_randjelovic@yahoo.com
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Abstract: In the winter time in Serbia, maize silage is the main ruminant feed. Therefore, managing maize silage is an important contributor to maintain the silage quality for livestock feed. In the study were evaluated the chemical composition, energetic and fermentation characteristics in whole-crop maize silage inoculated with different bacterial inoculants under field conditions in the commercial dairy farm, during the 2015. Three treatments were tested: negative control (untreated silage), a positive control (competitor inoculant) and Silko treatment (contains a mixture of 4 strains of *Lactobacillus plantarum* (LP1, LP2, LP3 and LP4). Maize is ensiled in the milk-wax grain maturity. After 90 days of ensiling, the maize silages were analyzed. The application of bacterial inoculants improved the chemical composition and energetic characteristics of silage. The inoculant Silko was more effective at improving the fermentation characteristics than competitor inoculant. Ash, cellulose, soluble N/TN, NH_3-N/TN, ADF, NDF, acetic acid and pH were significantly lower in Silko treatment than positive control. There were no differences in crude fat, crude protein, ME, NEL, lactic acid and butyric acid between the treated silages. Generally, the new product bacterial inoculant Silko proved in field trials its ability to support the ensiling process in maize. The main action of the bacterial inoculant Silko is performed in two ways: the reduced degradation of protein in silage and the improvement of the aerobic stability due to the lower pH, higher content of acetic acid than negative control.

Key words: chemical composition, energetic characteristics, fermentation parameters, *Lactobacillus plantarum*, maize silage

Introduction

The maize is a standard component of livestock diets. It can be harvested for grain and used in feed mixes for livestock, or entire plants can be harvested, chopped, and fermented for silage. Silage is made in order to feed animals in periods when feed supply is inadequate, either in terms of quantity or quality. The method of making maize silage is the simplest and acceptable to all of our farms. In Serbia, maize silage is one of the most important livestock feed especially in the winter. The starch, energy and intake characteristics of maize silage, together with its high dry matter yield potential, make it a good feed for beef cattle and sheep. *Mandić et al. (2013)* stated that the maize is very convenient crop for forage production because it has high production of green mass, energy content of dry matter and quality of biomass. Especially, ensiling of maize should be practiced when the plants of maize are damaged by frost, rain or drought, when reduction of grain yields is expected. However, maize silage requires high yield and harvest management, and good ensiling practices (rapid filling, thorough packing, perfect sealing and compression). The maize silage can be fermented under anaerobic conditions due to the native bacteria on plants; however, microbial additives reduce aerobic spoilage and help to maintain quality. The addition of bacterial and bacterial-enzyme inoculants is necessary in the initial stages of fermentation in order to achieve a rapid reduction of the pH, to avoid the occurrence and growth of harmful microorganisms, to avoid losses of dry matter and increase aerobic stability of silage *(Jatkauskas et al. 2013)*. Many researches showed that the adding microbial additives, improves the aerobic stability of maize silage and increases level of acetic acid *(Tabacco et al., 2009; Nkosi et al. 2011; Basso et al. 2013)*. In aerobic conditions yeasts and molds are developing, resulting in utilization of soluble carbohydrates and reducing nutritional value of silage, especially in warm weather so use of bacterial inoculants is necessary *(Ashbell et al., 2002)*. *Đorđević et al. (2011)* reported that bacterial-enzyme additives reduce fiber content and increase the concentration of sugar and lactic acid and digestibility of silage. *Bijelić et al. (2015)* concluded that bacterial-inoculants reduced crude protein content, NH_3-N/total nitrogen, acetic acid and pH value and increased the proportion of lactic acid relative to the acetic acid. *Weinberg et al. (2003)* reported that bacterial inoculants had effect on animal performance by increasing the nutritional value of the silage, and that some strains of lactic acid bacteria can survive in the gastric juice and have the role of buffer thus maintaining the activity of a cellulase enzyme and thereby increasing the digestibility. Also, *Acosta Aragón et al. (2012)* concluded that bacterial inoculants had positive effect on whole-crop maize silage quality, intake, feed energy utilization and performance of beef cattle. In the current market there are various bacterial inoculants containing different species and strains of bacteria. In most researches, their inclusion has provided positive effects on chemical or microbiological composition of the silage *(Wilkinson et al., 2003)* or on animal performance *(Contreras-Gouveia et al., 2010).* However, many researches showed that addition of homofermentative lactic acid bacteria (LAB) inoculants did not affect the fermentation parameter of maize silages *(Sucu and Filya, 2006; Sadeghi et al., 2012).* So, observing inconsistent results about the impact of inoculants on the quality of silage, constant developing of new microbial inoculants from native LAB in world is not surprising.

This study was conducted to evaluate the effects of bacterial inoculants on ensiling characteristics and nutritive value of whole-crop maize silage. Also, this study is intended to evaluate effects of new product Silko (produced in Serbia) compared to competitor product (positive control) available at the market.

Materials and Methods

The maize hybrid ZP 684 was harvested in August at the milk-wax stage of growth at 10mm theoretical length of cut using maize forage combine harvester. The silage mass was subdivided into three equal parts (negative control (untreated silage), a positive control (a competitor product available on the market) and Silko treatment) and ensiled in trench silos. The liquid inoculants were sprayed using a plant sprayer over the course of filling the silos at the rate of 5g t^{-1} fresh maize material. The inoculant Silko contains a mixture of four strains of *Lactobacillus plantarum* (LP1, LP2, LP3 and LP4). The number of colony forming units in inoculant is $1x10^{10}$ CFU/ml. After 90 days of ensiling, nine composite samples, three from each treatment were analyzed in laboratory. Composite sample included twelve samples which are collected with different locations in trench silo, and were mixed in a clean plastic bucket to form a composite sample weighing about 1.5 kg.

The content of dry matter (DM), ash, crude fat (CF), cellulose (Cell), neutral detergent fiber (NDF), acid detergent fiber (ADF), NH_3-N, lactic acid (LA), acetic acid (AA) and butyric acid (BA) and pH value were determined following the method described by *AOAC (2000).*

Total digestible nutrients value (TDN) and estimate dry matter digestibility (EDDM) calculated according to *NRC (2001)*, relative feed value (RFV) according to *Horrocks and Vallentine (1999)*, metabolic energy (ME) according to *Nauman and Bassler (1993)* and net energy for lactation (NEL) according to *Baležentienė and Mikulionien (2006)*:

TDN (%) = 87.84 – (%ADF × 0.70);

RFV (%) = Digestible Dry Matter (DDM) × Dry Matter Intake (DMI) × 0.775,

DDM (%) = 88.9 - (0.779 × %ADF) and DMI (%) = 120/(%NDF);

ME (MJ kg^{-1}) = 14.07 + 0.0206 × crude fat (g kg^{-1}) – (0.0147 × crude fibre (g kg^{-1}) – 0.0114 × crude protein (g kg^{-1}) ± 4.5 %;

NEL (MJ kg^{-1}) = 9.10 + 0.0098 × crude fat (g kg^{-1}) – 0.0109 × crude fibre (g kg^{-1}) – 0.0073 × crude protein (g kg^{-1}).
EDDM (%) = 88.9 – (0.779 × %ADF).

The experimental data were analyzed as a one-way ANOVA using Statistica version 10 and Duncan Multiple Range ($P < 0.05$) was used to compare means.

Results

Chemical composition

Data of ANOVA in Table 1 shows that both inoculants have highly significant effect on content of ash, crude fat, crude protein and cellulose. Also, Silko inoculant has significant effect on ADF and NDF. Ash was significantly lower in negative control (21.67 kg^{-1} DM) than Silko treatment (24.94 g kg^{-1} DM) and positive control (25.75 g kg^{-1} DM). Crude fat (17.49 g kg^{-1} DM) and crude protein (72.09 g kg^{-1} DM) were significantly lower in negative control than positive control (21.55 g kg^{-1} DM and 76.21 g kg^{-1} DM) and Silko treatment (20.60 g kg^{-1} DM and 78.27 g kg^{-1} DM). However, values of these parameters did not differ between positive control and Silko treatment. Cellulose was significantly higher in negative control (84.76 g kg^{-1}) than positive control (79.02 g kg^{-1} DM) and Silko treatment (74.41 g kg^{-1} DM). Value of this parameter was significantly lower in Silko treatment than positive control. ADF and NDF were significantly lower in Silko treatment (225.11 g kg^{-1} DM and 372.14 g kg^{-1} DM) than negative control (237.41 g kg^{-1} DM and 390.49 g kg^{-1} DM) and positive control (234.18 g kg^{-1} DM and 386.65 g kg^{-1} DM).

Table 1 Chemical composition of untreated silage and silage treated with bacteria inoculants

Item	Control	Positive control	Silko	M	F test
Dry matter (DM) (g kg^{-1})	362.27	374.50	391.30	376.02	ns
Ash (g kg^{-1} DM)	21.67^c	25.75^a	24.94^b	24.12	**
Crude fat (CF) (g kg^{-1} DM)	17.49^b	21.55^a	20.60^a	19.88	**
Crude protein (CP) (g kg^{-1} DM)	72.09^b	76.21^a	78.27^a	75.52	**
Cellulose (CEL) (g kg^{-1} DM)	84.76^a	79.02^b	74.41^c	79.40	**
Acid detergent fiber (ADF) (g kg^{-1} DM)	237.41^a	234.18^a	225.11^b	232.23	*
Neutral detergent fiber (NDF) (g kg^{-1} DM)	390.49^a	386.65^a	372.14^b	383.09	*

Means followed by the same letter within a column are not significantly different by Duncan's Multiple Range Test at the 5% level ($p \leq 0.05$), ** - significant at 1% level of probability, * - significant at 5% level of probability and ns - not significant

Energetic characteristics

Results showed that addition of inoculants did not affect total digestible nutrients value (TDN), relative feed value (RFV) and estimate dry matter digestibility (EDDM), Table 2. Their values were the lowest in negative control

(71.22%, 167.68% and 70.41%) and highest in Silko treatment (72.08%, 179.02% and 71.36%). Values of ME and NEL were significantly higher in positive control (12.48 MJ kg^{-1} and 7.89 MJ kg^{-1}) and Silko treatment (12.51 MJ kg^{-1} and 7.92 MJ kg^{-1}) than negative control (12.36 MJ kg^{-1} and 7.82 MJ kg^{-1}).

Table 2 Energetic characteristics of untreated silage and silage treated with bacteria inoculants

Item	Control	Positive control	Silko	M	F test
Total digestible nutrients value (TDN) (%)	71.22	71.45	72.08	71.58	ns
Relative feed value (RFV) (%)	167.68	169.95	179.02	172.22	ns
Metabolic energy (ME) (MJ kg^{-1})	12.36[b]	12.48[a]	12.51[a]	12.45	**
Net energy for lactation (NEL) (MJ kg^{-1})	7.82[b]	7.89[a]	7.92[a]	7.88	**
Estimate dry matter digestibility (EDDM) (%)	70.41	70.66	71.36	70.81	ns

Means followed by the same letter within a column are not significantly different by Duncan's Multiple Range Test at the 5% level ($p \leq 0.05$), ** - significant at 1% level of probability and ns - not significant

Fermentation parameters

Data of ANOVA in Table 3 shows that silage inoculants have significant effect on all fermentation parameters. The content of soluble N/TN (370.96 g kg^{-1} TN) and NH_3-N/TN (38 g kg^{-1} TN) were lower in Silko treatment compared to negative control (381.63 g kg^{-1} and TN 45.16 g kg^{-1} TN) and positive control (494.98 g kg^{-1} TN and 51.45 g kg^{-1} TN). The lactic acid (70.08 g kg^{-1} DM) and acetic acid (5.23 g kg^{-1} DM) were significantly lower in negative control than silage treated with inoculants. Lactic acid was similar in positive control (82.57 g kg^{-1} DM) and Silko treatment (82.88 g kg^{-1} DM). Contrary, acetic acid was lower in Silko treatment (11.17 g kg^{-1} DM) than positive control (12.95 g kg^{-1} DM). The butyric acid (0.28 g kg^{-1} DM) and pH value (4.26) were significantly higher in negative control than positive control (0.11 g kg^{-1} DM and 3.97) and Silko treatment (0.09 g kg^{-1} DM and 3.82).

Table 3 Fermentation parameters of untreated silage and silage treated with bacterial inoculants

Item	Control	Positive control	Silko	M	F test
pH	4.26[a]	3.97[b]	3.82[c]	4.02	**
Soluble N/TN (g kg^{-1} TN)	381.63[a]	494.98[b]	370.96[a]	415.86	**
NH_3-N/TN (g kg^{-1} TN)	45.16[b]	51.45[a]	38.00[c]	44.87	**
Lactic acid (LA) (g kg^{-1} DM)	70.08[b]	82.57[a]	82.88[a]	78.51	**
Acetic acid (AA) (g kg^{-1} DM)	5.23[c]	12.95[a]	11.17[b]	9.78	**
Butyric acid (BA) (g kg^{-1} DM)	0.28[b]	0.11[a]	0.09[a]	0.16	**

Means followed by the same letter within a column are not significantly different by Duncan's Multiple Range Test at the 5% level ($p \leq 0.05$), ** - significant at 1% level of probability

Discussion

Inoculants did not cause significant changes of dry matter content. Similar results found *Bijelić et al. (2015)*. Dry matter content in investigated silages ranged from 362.27 g kg^{-1} (negative control) to 391.30 g kg^{-1} (Silko treatment). *Loučka (2010)* pointed that optimal content of DM for maize silage is 280-340 g kg^{-1}, but in our silages DM was higher. The higher dry matter content can be explained by the fact that is during the drying process of samples, there was a loss of moisture and volatile organic substances. During the drying process of silage samples, heat drying could result in losses of volatile substance, such as short-chain organic acids and alcohols *(McDonald et al., 1991)*. The content of ash in investigated silages ranged from 21.67 g kg^{-1} DM (negative control) to 25.75 g kg^{-1} DM (positive control). It is ideally because ash values were lower than 85 g kg^{-1} DM and indicate that silages are not contaminated with soil. The higher contents of ash in inoculated silages result is metabolism of inoculated strains bacteria. The inoculated silage had significantly higher crude fat and crude protein content. *Nkosi et al. (2011)* reported similar results. Contrary, *Bijelić et al. (2015)* found that crude protein content decreased with the addition of the bacterial inoculant. The studied inoculants significantly decreased cellulose content of maize silage compared to the untreated silage, especially Silko. This decrease in cellulose content can be due to ability of *Lactobacillus* species to produce cellulose enzyme, *Sadiya and Ibrahim (2015)*. Contrary, *Dinić et al. (2013)* concluded that bacterial inoculant did not affect cellulose content of maize silage, but *Konca et al. (2015)* found that LAB inoculation decreased cellulose content of sunflower silages. The silage inoculated with bacteria inoculants reducing fiber fractions (ADF and NDF) compared to negative control. Values of ADF and NDF are important because they relate to the ability of an animal to digest the forage. As ADF increases, digestibility of forage decreases, while NDF decreases, the dry matter intake increases. The ADF value refers to the cell wall portions of the forage that are made up of cellulose and lignin, while NDF value is the total cell (ADF fraction plus hemicellulose). In Silko treatment, content of ADF and NDF significantly reduced which indicates that part of the fiber was solubilized. NDF is reduced because of the increased degradation of hemicellulose. Generally, favorable anaerobic conditions reducing ADF and NDF. According to *NRC (2001)*, maize silage with over 400 g dry matter contains TDN 65.4%, NDF 445g kg^{-1} DM, ADF 275 g kg^{-1} DM and ash 40 g kg^{-1} DM. In our study, the values for NDF and ADF slightly lower.

Silages treated with inoculants have higher ME and NEL than untreated silage. However, TDN, RFV and EDDM values did not differ between treatments, although the highest values were in Silko treatment. *Dinić et al. (2013)* found that applying bacterial inoculant to maize silage increased the NEL and RFV values.

Maize silages inoculated with inoculants have a lower pH and content of butyric acid, and higher content of lactic acid and acetic acid compared to negative control. For whole-crop maize preservation, pH between 3.8 and 4.5 is considered to be beneficial. In our study the pH ranged from 3.82 (Silko treatment) to 4.26 (negative control) which is indicative of well-preserved silage. The content of soluble N/TN and NH_3-N was significantly lower in Silko treatment than positive control. These values were highest in positive control which can be associated with a slightly higher pH than Silko treatment. Also, this indicates that the proteins are extensively degraded. Studied silages have a less content of NH_3-N than the limit values 7-10% *(Đorđević and Dinić, 2003)*. Generally, treated silages with inoculant Silko contains more of the protein in an intact form that can be utilized directly by the animal. The studied silages had satisfactory content of lactic acid (>6.5%), indicating a good fermentation. Silages treated with inoculants have higher concentrations of acetic acid and lower concentrations of butyric acid than untreated silage. The high levels of acetic (> 3 - 4%) and butyric acid (> 0.5%) are undesirable because indicates poorly fermented silage. The studied silages have the lower content of acetic and butyric acid than these values and indicate good fermented silages. The significantly lower values of lactic acid and acetic acid and the higher value of butyric acid were recorded in negative control. Values of lactic acid and butyric acid did not differ between the inoculants tested, while content of acetic acid was significantly higher in positive control. Acetic acid has anti-fungal properties, reduces aerobic spoilage of silage and growth of molds and yeasts. Accordingly, application of the tested inoculant can have these effects, in a way of increasing acetic acid content. Bacterial inoculant Silko was effective to enhance aerobic stability of silages due to higher acetic acid production which have antimycotic properties. The decrease in the pH values of fermentation in treated silages may be justified by the increase in the concentration of lactic acid. Also, *Đorđević et al. (2016)* reported that Silko inoculant increases lactic acid and acetic acid, and decreases butyric acid production of alfalfa silage compared to control. The lower content of butyric acid indicates that investigated silages did not content Clostridia spores which degrade lactic acid to butyric acid, and are results of contamination of fresh plant material with soil. It can be concluded that field trials showed good ensiling practices (rapid filling, packing thorough, perfect sealing and compression). Generally, fermentation characteristics in treated silages with Silko indicate good silage quality.

Conclusion

This study showed that application of bacterial inoculants of whole-crop maize during ensiling may improve the silage quality compared to untreated silage. However, inoculant Silko was more effective at improving the fermentation

characteristics than competitor inoculant. The content of ash, cellulose, soluble N/TN, NH_3-N/TN, ADF, NDF, pH and acetic acid differences were found between the positive control (competitor inoculant) and Silko inoculant. These values were significantly lower in Silko treatment. Silage inoculated with Silko were lowest content of NH_3-N, which is indicative of well-preserved silage this effect arose as a result of the pH reduction with inoculation which inhibits protein degradation in silages. However, no differences in crude fat, crude protein, metabolic energy, net energy for lactation, lactic acid and butyric acid between inoculant treatments. Results showed that inoculant Silko is efficient to improve chemical composition and energetic characteristics and reduced fermentative losses of maize silage. New inoculant Silko is high-performance inoculant for maize silage.

Uticaj *Lactobacillus plantarum* inokulanata na kvalitet silaže kukuruza

Snežana Đorđević, Violeta Mandić, Dragana Stanojević, Nataša Jovanović Ljesković

U zimskom periodu u Srbiji, silaža kukuruza je glavna hrana za preživare. Zbog toga je postupak proizvodnje silaže važan faktor u očuvanju kvaliteta silaže za ishranu zivotinja. U studiji su ocenjeni hemijski sastav, energetske i fermentacione karakteristike silaža od celih biljaka kukuruza inokulisanih različitim bakterijskim inokulantima u terenskim uslovima na komercijalnoj farmi goveda u 2015. godini. Tri tretmana su testirana: negativna kontrola (netretirana silaža), pozitivna kontrola (konkurentski proizvod) i Silko tretman (sadrži mešavinu 4 soja *Lactobacillus plantarum* (LP1 LP2, LP3 i LP4)). Kukuruz je siliran u fazi mlečno-voštane zrelosti zrna. Silaža je analizirana 90 dana nakon siliranja. Bakterijski inokulanti su poboljšali hemijski sastav i energetske karakteristike silaže. Inokulant Silko bio je efikasniji u poboljšanju fermentacionih karakteristika u odnosu na konkurentski proizvod. Sadržaj pepela, celuloze, rastvorljivog i amonijačnog azota u ukupnom azotu, ADF, NDF, sirćetne kiseline i pH značajno su niži u Silko tretmanu nego u pozitivnoj kontroli. Nije bilo razlike u sadržaju sirove masti, sirovih proteina, ME, NEL, mlečne i buterne kiseline između tretiranih silaža. Generalno, novi proizvod bakterijski inokulant Silko pokazao je da u poljskim ogledima ima sposobnost da podrži proces siliranja kukuruza. Delovanje bakterijskog inokulanta Silka vrši se na dva načina: smanjena degradacija proteina u silaži i poboljšana aerobna stabilnost zbog nižeg pH i većeg sadržaja sirćetne kiseline u poređenju sa negativnom kontrolom.

Ključne reči: hemijski sastav, energetske karakteristike, parametric fermentacije, *Lactobacillus plantarum*, silaža kukuruza

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