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Inoculation of whole-plant maize with viable lactic acid bacteria: effects on silage fermentation, aerobic stability and performance of dairy cows

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Abstract

The study was aimed to evaluate the effect of the addition of a lactic acid bacteria (LAB) inoculant to whole-plant maize at ensiling on the quality, aerobic stability of the silage as well as the intake, milk production and milk composition of Lithuanian black and white dairy cows fed inoculated or not inoculated maize silage diets. The inoculant contained *Lactobacillus plantarum*, *L. brevis* and *L. kefiri* as well as carrier inulin. Maize was ensiled in two silo trenches with or without the inoculant. Chemical and microbiological analyses were carried out on a representative sample of the 5 replicates for each control and inoculated silage at day 92 of storage. At day 92 of ensiling, the silage was exposed to air to determine the aerobic stability. Inoculation caused a reduction in pH value, increased lactate and propionate concentrations and decreased ammonia nitrogen, ethanol and butyrate concentrations. Yeast and mould contamination was reduced in the inoculated silage, which led to an increased aerobic stability compared to the not inoculated one. The inoculated silage had a higher energy concentration, and cows fed on it produced higher fat and energy corrected milk yield from day 69 until end of the experiment (day 92). Milk from cows fed the inoculated silage had a higher fat percentage, similar contents of protein and a lower number of somatic cell counts compared with that from cows fed control. Feed efficiency was improved in cows fed the inoculant treated silage compared to the conventionally produced silage.

Keywords: aerobic stability, fermentation, inoculant, trench silo, feed efficiency.

Introduction

Forages account for up to two thirds of feed for dairy cattle, thus the quality of forage is an important determinant of dairy cows' performance. Production of high-quality silage from whole-plant maize and high milk yield from cows depend on the success of ensiling. Silage fermentation products and their ratio together with yeast and mould population show the ability to inhibit the activity of undesirable microorganisms under anaerobic conditions and to produce high quality silage (Muck, 2012).

When a silo trench is opened for feeding, silage coming in contact with the air causes development of yeast and mould, a rise in temperature and aerobic deterioration of silage. Due to aerobic deterioration of silage quality, sensory properties and nutritive value decline (Dolci et al., 2011; Schmidt, Kung, 2010; Muck, 2012). Richard et al. (2009) and Weiss et al. (2016) suggest that badly produced or aerobically spoiled silage decrease nutritive value, feed intake and productivity of dairy cows, and also initiates the growth of undesirable microorganisms, which cause mycotoxin synthesis. To stimulate organic acid production and to attain rapid decrease of acidity (pH) microbial additives containing lactic acid bacteria (LAB) are frequently used. It was found that heterofermentative LAB can reduce aerobic deterioration and improve hygienic quality of the silage (Tabacco et al., 2011). For the inoculation of the silage, relevant properties are the ability of LAB strains to shift fermentation and to reduce population of undesirable moulds, most yeasts and some harmful bacteria (Wilkinson, Muck, 2019; Amaral et al., 2020; Guan et al., 2020).

Whole-plant maize silage has become the predominant forage used in dairy cattle diets worldwide, because maize is one of the most productive plants and a good energy source for livestock (Iqbal et al., 2014). The growing use of maize, its nutritive value and positive environmental effects compared to other crops show the importance of its silage use for dairy cattle. However,

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there is a lack of information on the specificity of its production and use by farmers.

The objective of this experiment was to evaluate the magnitude of the effects of homofermentative and heterofermentative LAB blend inoculation on whole-plant maize silage quality and deterioration under aerobic conditions. Herewith, it was hypothesized that inoculation of maize forage with viable LAB would improve the productivity of dairy cows and milk composition together with better nutrient utilization in dairy cows.

Materials and methods

Silage preparation and treatments. Whole-plant maize (*Zea mays* L., cultivar 'Baxxos FAO 200') was cultivated at the Experimental Farm, Institute of Animal Science of Lithuanian University of Health Sciences in Baisogala, Radviliškis district, and was ensiled, when the grain was at a dough line stage of maturity. On 14 October 2018, maize (355 g kg^{-1} DM) was harvested with a forage harvester Massey Ferguson 5130 (Germany) adjusted to achieve a ≈10 mm theoretical cut length. Maize was treated either with chlorine free water (control, CTL) or with inoculant suspension BioStabil (BST) containing *Lactobacillus plantarum*, *L. brevis* and *L. kefiri* Biomin® BioStabil (Biomin GmbH, Austria). The suspension and water were applied on the forage chopper with an applicator to provide 10^5 colony forming units (cfu) of lactic acid bacteria (LAB) per g of fresh forage (1×10^5 cfu g^{-1} FF). Chemical and microbial analyses of fresh maize were performed before the inoculant suspension had been applied on a representative sample of the 5 replicates. Fine-cut whole-plant maize was delivered and ensiled into two concrete silo trenches with a capacity of 200 tons each. To determine fresh (FM) and dry matter (DM) loss, 5 control bags (made from four layers of cheesecloth) were filled with 1 kg with either of CTL or BST fresh forage and placed into corresponding silo trench. The ensiling process was finished within 48 h, and ensiled maize was sealed with plastic cover and weighted down with sandbags. Both CTL and BST silo trenches were opened at day 92 of storage, core sampled (5 samples per treatment and 2 analyses per sample) using a 50 mm diameter silage drill, and the chemical and microbiological analyses were carried out. Throughout the feeding trial, representative weekly samples of the CTL and BST silage were taken directly after the silage was taken from the trench for feeding. Analyses of nutrient and fermentation products were carried out at the Chemical Laboratory of the Institute of Animal Science of Lithuanian University of Health Sciences, and microbial counting was performed at National Food and Veterinary Risk Assessment Institute, Lithuania. Samples of maize plant and silage were processed, and dry matter, nutrient content and acidity of fermentation products (pH), contents of lactic, acetic, propionic and butyric acids, ammonia nitrogen (NH_3-N) and alcohols (only on silage) were determined as described by Jatkauskas et al. (2013). At day 92 after ensiling, silages were subjected to an aerobic stability test at a constant (20–21°C) temperature lasting 11 days. Temperature development inside silo trench was measured as described by Jatkauskas et al. (2013).

Calculations. Silage dry matter (DM) content was corrected for volatile compounds lost during the oven drying. Dry matter corrected for volatiles (DMC) was calculated according to the method described by Weissbach and Strubelt (2008):

$$DMC = DM + (1.05 - 0.059 \times pH) \times FA + 0.08 \times LA + 0.77 \times PD + 0.87 \times BD + 1.0 \times OA,$$

where FA is fatty acids ($C_2 \dots C_6$), LA – lactic acid, PD – 1,2-propanediol, BD – 2,3-butanediol, OA – other alcohols ($C_2 \dots C_4$).

Fresh matter loss during fermentation was calculated by measuring difference in weights of fresh forage and fresh silage in control bags (predicted to 1 kg). Dry matter loss was calculated having DM content of the forage and silage using the following equation:

$$\text{Dry matter loss} = (\text{DM at ensiling} - \text{DMC silage}) / \text{DM at ensiling}.$$

Feeding trial. For determining the effect of inoculant treatment on feed intake and milk yield, the feeding experiment with Lithuanian black and white dairy cows was conducted as a complete randomized block design. The experiment was conducted in compliance with the requirements of the guidelines of the Declaration of Helsinki and approved by the Law of the Republic of Lithuania on Animal Care, Housing and Use (TAR, 2015).

Thirty-six multiparous cows with an average of 120 ± 11.8 days in milk were blocked in pairs according to individual live weight, lactation number, milk yield (previous month) and body condition score. After three weeks' adaptation period, when all 36 cows were fed CTL silage to adapt animals to diet changes, the animals were divided into two groups with 18 replicates (cows) per treatment (group). During the experimental period (92 days), one group of cows was fed CTL silage and the other – BST silage. Silages were offered for *ad libitum* intake twice daily, and the amount of offered feed was adjusted daily to allow refusals of 10% (FM basis). The amount of silage offered was increased for the next day, if there were no refusals; otherwise, the amount offered was kept the same. To estimate dry matter intake (DMI), the amount of offered and refused feed was measured once weekly on two consecutive days. A compound feed (6 kg cow day^{-1}) was top dressed twice daily after each milking (07:00 and 17:00 h) for both groups.

Ingredients and chemical composition of the compound feed are shown in Table 1. Experimental diets were calculated to meet nutrient requirements (Agroscope, 2016) and based on maize silage as forage source only. The animals were housed in tie-stalls in individual pens. To avoid cross-contamination of diets, animals of each treatment were tie-stalled across the feeding bunk. Pens were equipped with individual feeding troughs and water bowls. Temperature and humidity were kept between 10–15°C and 70%, and light was provided 24 h. The cows were observed at least once daily for signs of diseases.

Samples of the given and the rest of the silage were taken every day and frozen. These samples were pooled for each week and were analysed for chemical composition to calculate nutrient value of consumed silage. Cows were milked twice daily (06:00 and 16:00 h), and individual milk yield was recorded. A composite sample (morning and evening milking) was analysed once weekly for the content of fat, protein, lactose, urea and somatic cell count (SCC). The samples were analysed at the accredited laboratory of milk testing "Enterprise Pieno Tyrimai" (Lithuania). Milk fat, protein, lactose and urea contents were determined using infrared milk analyser (LactoScope FTIR). The SCC analysis was determined by somatic cell counter SomaScope Smart (Perkin Elmer, USA).

Table 1. Chemical composition and estimated nutrient value of compound feed top-dressed twice daily on experimental silage to both treatment groups

Ingredients %	
Barley	27
Wheat	15
Soybean meal	25
Rapeseed expeller	15
Dried molasses sugar beet pulp	15
Mineral and vitamin supplement	3
Chemical composition	
Dry matter (DM) g kg^{-1}	891
Crude protein (CP) g kg^{-1} DM	262
Crude fat g kg^{-1} DM	33.5
Crude fibre (CF) g kg^{-1} DM	77.3
Neutral detergent fibre (NDF) g kg^{-1} DM	181
Water soluble carbohydrates (WSC) g kg^{-1} DM	149
Crude ash g kg^{-1} DM	51.5
Estimated metabolizable energy MJ kg^{-1} DM	12.8
Estimated netto energy lactation (NEL) MJ kg^{-1} DM	8.07

Statistical analysis. The data were statistically processed using analysis of variance (ANOVA) to test for the effect of silage treatments with *Genstat* (Payne et al., 2011). For the feed intake and milk yield, each animal within a group was considered an experimental unit. To determine statistical differences between the treatments, the Fisher's least significant difference (LSD) procedure at the 5% significance level was used.

Results

Characterization of whole-plant maize crop prior to ensiling. The chemical composition of pre-ensiled whole-plant maize: dry matter (DM) – 354.7 g kg^{-1}, crude protein (CP) – 101.2 g kg^{-1} DM, water soluble carbohydrates (WSC) – 79.3 g kg^{-1} DM, and acidity (pH value) corresponded to dough stage of maturity of maize forage. Buffer capacity (BC) (25.42 mEq 100 g^{-1} DM) and the calculated fermentability coefficient (FC) (38.0) indicated that whole-plant maize was moderately difficult to ensile (DLG, 2018). The number of epiphytic lactic acid bacteria (LAB), mould and yeast (4.33, 4.34 and 5.03 $\log_{10}$ cfu g^{-1} FF, respectively) was typical of the microbial contamination in Lithuanian climate conditions.

Nutrient content, fermentation pattern and microbial properties of the silages at opening are presented in Table 2. Inoculation of whole-plant maize forage with the viable LAB strains affected the silage nutrient composition and fermentation profile. After 92 days of fermentation period, the inoculated (BST) silage had significantly higher DMC content (339 *vs* 325 g kg^{-1}) and significantly lower DMC loss (51.4 *vs* 87.4 g kg^{-1} DM) compared to spontaneously fermented silage. The greatest effect was observed for fibre fractions, as crude fibre (CF) and neutral detergent fibre (NDF) content was decreased ($P < 0.01$), while the concentrations of acid detergent fibre (ADF), CP and WSC remained unaffected by inoculation. Viable LAB BST treatment changed fermentation profile; this was indicated by the decreased ($P < 0.01$) pH and the proportions of organic acids at silo trench opening compared with the not inoculated (CTL) silage. BST silage contained higher ($P < 0.01$) concentrations of lactic and propionic acids, numerically higher concentration of acetic acid and lower ($P < 0.01$) concentrations of butyric acid, alcohols and ammonia nitrogen compared to CTL silage. Inoculant treatment resulted in increased metabolizable energy and netto energy lactation (NEL) content ($P < 0.01$). Inoculation suppressed yeast and mould growth ($P < 0.01$) and improved aerobic stability by 108 hours (2.6 days).

During aerobic exposure the CTL silage took 84 h to reach a temperature of more than 3°C over ambient, while the BST silage reached a temperature greater than 3°C over ambient at hour 216 of aerobic exposure. Moreover, the CTL silage reached a maximum temperature of 28°C, while the maximum temperature measured in the BST silage was 24°C (Figure 1).

Table 2. Nutrient content, fermentation profile and microbial population of maize silage as effected by inoculation

	Treatment			
	CTL	BST	SE	*P*-value
Dry matter corrected for volatiles (DMC) g kg^{-1}	325 a	339 b	2.060	0.01
Crude protein (CP) g kg^{-1} DM	101a	103 a	0.640	0.107
Crude fibre (CF) g kg^{-1} DM	220 a	201 b	2.226	0.01
Neutral detergent fibre (NDF) g kg^{-1} DM	438 a	413 b	4.486	0.01
Acid detergent fibre (ADF) g kg^{-1} DM	256 a	252 a	4.490	0.646
Water soluble carbohydrates (WSC) g kg^{-1} DM	5.66 a	5.73 a	0.186	0.843
Crude fat g kg^{-1} DM	29.1 a	29.2 a	0.275	0.840
Crude ash g kg^{-1} DM	62.7 a	59.1 b	0.644	0.01
Lactic acid g kg^{-1} DM	45.8 a	63.3 b	1.941	0.01
Acetic acid g kg^{-1} DM	19.5 a	21.07 a	0.601	0.181
Butyric acid g kg^{-1} DM	0.09 a	0.02 b	0.010	0.01
Propionic acid g kg^{-1} DM	0.14 a	0.25 b	0.015	0.01
Alcohols g kg^{-1} DM	9.52 a	5.26 b	0.475	0.01
Ammonia nitrogen g kg^{-1} N	54.6 a	39.6 b	1.574	0.01
Acidity (pH)	3.86 a	3.74 b	0.015	0.01
Metabolizable energy MJ kg^{-1}	11.2 a	11.4 b	0.026	0.01
Netto energy lactation (NEL) MJ kg^{-1}	6.60 a	6.82 b	0.026	0.01
DMC g kg^{-1} DM	87.4 a	51.4 b	3.680	0.01
Yeast, log cfu g^{-1} FF	3.54 a	2.45 b	0.913	0.01
Moulds, log cfu g^{-1} FM	3.22 a	2.18 b	0.178	0.01
Aerobic stability, hours	90 a	198 b	2.267	0.01

Note. FF – fresh forage; CTL – not inoculated (control), BST – inoculated with BioStabil; SE – standard error; a, b – within a row mean values followed by different letter differ significantly.

Feed intake and dairy cow performance. The nutrient composition of consumed maize silage and feed intake by dairy cows are presented in Table 3.

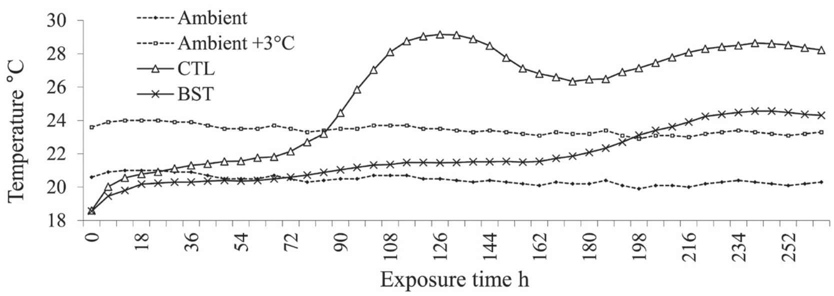


Figure 1. Temperature changes inside not inoculated (CTL) and inoculant treated (BST) silage over 11 days of aerobic exposure

Table 3. Nutrient composition of consumed maize silage and feed dry matter intake by dairy cows (92 days feeding period)

	Treatment			
	CTL	BST	SE	*P*-value
DM (dry matter) g kg^{-1} as fed	327.5 a	342.4 b	2.25	0.01
Crude protein (CP) g kg^{-1} DM	100.4 a	102.7 a	0.69	0.095
Crude fibre (CF) g kg^{-1} DM	215.7 a	194.6 b	2.43	0.01
Neutral detergent fibre (NDF) g kg^{-1} DM	436.6 a	408.4 b	5.07	0.01
Acid detergent fibre (ADF) g kg^{-1} DM	251.7 a	248.4 a	4.78	0.736
Water soluble carbohydrates (WSC) g kg^{-1} DM	5.99 a	6.13 a	0.19	0.716
Crude fat g kg^{-1} DM	29.2 a	29.2 a	0.29	0.92
Crude ash g kg^{-1} DM	62.7 a	58.5 b	0.71	0.01
Netto energy lactation (NEL) MJ kg^{-1} DM	6.67 a	6.89 b	0.03	0.01
			Feed intake	
Silage kg^{-1} DM day	12.00 a	12.21 a	0.17	0.532
Silage NEL MJ^{-1} day	80.5 a	83.7 a	1.13	0.161
Compound feed kg^{-1} DM day	5.35 a	5.35 a	0.00	0.000
Total diet intake kg^{-1} DM day	17.4 a	17.6 a	0.16	0.527
Total NEL intake MJ^{-1} day	123.7 a	126.9 a	1.13	0.161

Note. CTL – not inoculated (control), BST – inoculated with BioStabil; SE – standard error; a, b – within a row mean values followed by different letter differ significantly.

The chemical composition of the consumed whole-plant maize silage was close to that of silage analysed at silo trench opening (Table 2). BST silage had a numerically higher (by 4.5%) dry matter content and significantly higher estimated NEL content compared to CTL one. The cows given BST silage ate numerically more (by 1.8%) dry matter than those fed the CTL one. NEL intake with consumed silage was numerically higher (by 3.9%) for dairy cows offered BST silage compared to CTL one. The intake of concentrate was restricted and did not differ between the treatments. The present experimental data revealed that feeding BST silage numerically increased (by 0.7 kg day^{-1}) average milk yield (Table 4).

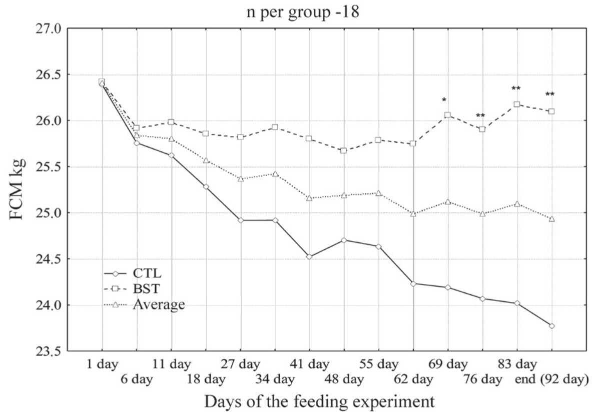
Table 4. Milk production and composition of dairy cows fed either not inoculated (CTL) or inoculant treated (BST) maize silage (92 days feeding period)

	Treatment			
	CTL	BST	SE	*P*-value
	Production kg day^{-1} cow			
Milk	24.4 a	25.1 a	0.37	0.275
Milk fat	1.00 a	1.06 a	0.02	0.059
Milk protein	0.77 a	0.80 a	0.01	0.194
Milk lactose	1.11 a	1.15 a	0.02	0.242
FCM[2]	24.8 a	25.9 a	0.29	0.117
ECM[1]	26.47 a	27.69 a	0.33	0.111
	Components %			
Milk fat	4.12 a	4.20 b	0.02	0.05
Milk protein	3.16 a	3.18 a	0.01	0.309
Milk lactose	4.56 a	4.58 a	0.01	0.564
Milk urea mg %	29.2 a	30.0 a	0.25	0.096
SCC ($\times 10^3$ mL^{-1})	155 a	114 b	6.21	0.01
	Feed efficiency			
Milk yield and DMI ratio	1.40 a	1.43 b	0.009	0.01
FCM yield and DMI ratio	1.43 a	1.47 b	0.012	0.01
ECM yield and DMI ratio	1.52 a	1.57 b	0.017	0.01
FCR, NEL and ECM yield ratio	4.99 a	4.90 a	0.023	0.01

Note. FCM – fat corrected milk, ECM – energy corrected milk; SCC – somatic cell count; DMI – dry matter intake; FCR – feed conversion ratio, NEL – netto energy lactation; [1] – ECM = 0.327 × milk yield (kg day^{-1}) + 12.95 × fat yield (kg day^{-1}) + 7.2 × protein yield (kg day^{-1}), [2] – 4% FCM = 0.4 × milk yield (kg day^{-1}) + 15 × fat yield (kg day^{-1}) (Tyrrell, Reid, 1965); SE – standard error; a, b – within a row mean values followed by different letter differ significantly.

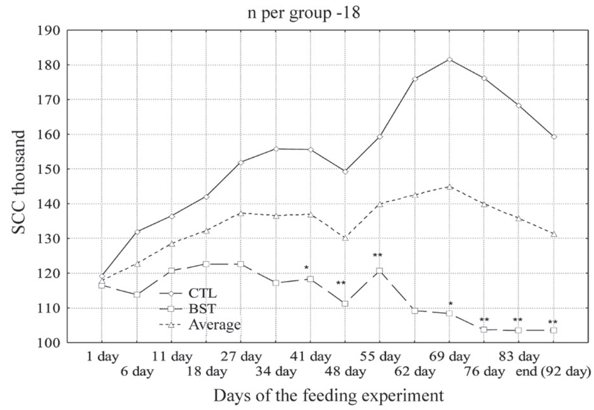
Milk of dairy cows fed BST silage contained significantly higher percentage of fat compared to that of cows fed CTL one. Therefore, when evaluating milk yield over time, significantly higher, fat corrected milk yield for cows fed BST silage from day 69 until end of the 92 days feeding period was revealed (Figure 2). No significant differences were observed for the milk lactose, milk protein and urea content between the treatments. BST silage improved hygienic quality of milk. Milk from cows fed BST silage contained a significantly lower number of somatic cells, when compared with that of cows fed CTL one. Starting from day 31 until end of feeding period, somatic cell count was significantly lower in the milk of cows fed BST silage (Figure 3).

Feed efficiency expressed as kilograms of milk, fat corrected milk, energy corrected milk produced per kilogram of DMI was significantly higher for dairy cows fed BST silage compared to those fed CTL one (Table 4). Feed conversion ratio expressed as NEL per 1 kg energy corrected milk was improved by cows fed BST silage compared with CTL one.



* and ** – significant at 0.05 and 0.01, respectively

Figure 2. Dynamic of fat corrected milk (FCM) yield of dairy cows fed either not inoculated (CTL) or inoculant treated (BST) maize silage



* and ** – significant at 0.05 and 0.01, respectively

Figure 3. Somatic cell count (SCC) in milk of dairy cows fed either not inoculated (CTL) or inoculant treated (BST) maize silage

Discussion

Silage fermentation profile. The main two factors affecting silage quality are the microbial population and the forage chemical composition, which determines the buffer capacity and fermentation coefficient of forage (Muck, 2013). In our experiment, whole-plant maize forage was of the expected quality, and ensiling conditions were homogenous between the treatments. The calculated fermentability coefficient (FC) was medium; consequently, the whole-plant maize was considered to be moderately difficult to ensile. During the fermentation volatile compounds are produced, which will often lower the DM content compared to fresh forage, which explains the higher DM in fresh forage compared to ensiled one (Kristensen et al., 2010).

Higher amounts of lactic acid and thereby reduced pH suggest that inoculation supported a fast and steady colonization with LAB, which is mandatory for the anaerobic ensiling process. It can be suggested that trough the dominance of *L. plantarum* the silage underwent a prevalent homofermentative fermentation, as shown by relatively higher ratio of lactic acid to acetic acid compared to not inoculated silage (Daniel et al., 2015). A fast rate of the fermentation and higher decline in pH value of the silage supported by homofermentative LAB have been reported in some studies (Muck, 2010; Arriola et al., 2011). As hypothesized, inoculation can reduce DM loss during anaerobic storage and, therefore, improve production efficiency, as it is reported that reduced anaerobic fermentation losses result in higher energy value by 0.1 to 0.2 MJ kg^{-1} DM NEL (Spiekers et al., 2009).

Energy loss and protein degradation are even higher, when stability is low, and aerobic fermentation by yeasts metabolizing lactate to ethanol is induced leading to decreased nutrient value of silage. Results of our experiment indicate that undesired aerobic fermentation by epiphytic microorganism was limited, and spoilage of yeasts and moulds was reduced, as their fermentation end products like ethanol, butyrate and ammonia nitrogen levels were lowered in BST silage compared to the CTL one.

According to Kung and Ranjit (2001), *L. plantarum* is a strain selected for fast growth, production of lactic and acetic acid and ability to suppress strains of yeast that cause spoilage in maize silage and are usually the initiator of heating. Morel et al. (2018) stated that low aerobic stability during feed-out can lead to self-heating and spoilage of the silage. Usage of heterofermentative LAB strains like *L. buchneri* are widely discussed to produce higher amounts of acetic acid trough the ability to ferment lactic acid to acetic acid and 1,2 propanediol (Daniel et al., 2015).

As mentioned earlier, acetic acid is a desired fermentation product due to its antifungal properties beneficial for aerobic stability (Muck, 2010). In the recent experiment, no effects could be observed on the production of acetic acid, although aerobic stability was improved in the BST silage to great extent. It can be suggested that besides desired production of acetic acid LAB strains have a more differentiated fermentation profile supporting silage aerobic stability, as silage fermentation is a complex multistage process. With reduced growth of deleterious epiphytic bacteria, DM and nutrient losses were minimized in the BST silage by 41% compared to the CTL one.

Large amounts of CO_2 produced trough undesired fermentation are leading to losses of dry matter as carbon will be volatilized (Kung, Ranjit, 2001). Reduced spoilage positively contributes to DM recovery, as was also shown by Borreani et al. (2018). There are only a limited number of studies that have been conducted with the combination of both heterofermentative and homofermentative LAB, and the results have been discussed controversially (Muck, 2010; Arriola et al., 2011). A meta-analysis carried out by Oliveira et al. (2017) suggests that the usage of two or more LAB strains applied together may have synergistic effects compared to single strains alone. In addition, they concluded that combined inoculants might be more effective against moulds induced by the production of antifungal compounds. Inoculation of silage enhanced CF and NDF degradation, although the effect has not yet been fully understood.

However, recently *L. buchneri* and *L. brevis* have been described to produce ferulate esterase – an enzyme, which increases cell wall degradation releasing more soluble carbohydrates from plants for fermentation or usage by rumen bacteria. Further, it is described that LAB have positive effects on digestibility and, therefore, potentially on performance (Nsereko et al., 2008). These data agree with our findings showing reduced CF and NDF in the BST silage. As no effect was observed on concentration of WSC but lactate concentration was increased, it can be suggested that the second condition prevailed, and formation of lactic acid was prolonged. The microbial analysis and aerobic stability test results indicated that inoculant treatment was efficient to prevent yeast and fungal growth and temperature increment during aerobic storage of maize silage. Muck (2012) reported that if the yeast population decreases during silage fermentation or during feed out phase then aerobic stability increases.

Feed intake and dairy cow performance. To enhance animal performance through an improved fermentation profile and hygiene of silage, inoculants are used. Moreover, Weinberg et al. (2003) found that live LAB silage inoculants can enhance ruminant performance and increase feed efficiency. The improvement in production and feed efficiency in dairy cows fed inoculated silage suggests a possible probiotic effect of the viable LAB received by cows with silage (Weinberg et al., 2004). As described by Huhtanen et al. (2013), it has been shown that extent and fermentation products of the silage influences milk yield and chemical composition, and further that improved silage fermentation quality positively affects DMI and performance. Different LAB strains have been described to improve DMI; however, results reported in literature were not consistent and cannot be supported by results found in the recent experiment.

It can be suggested that differences may have been too low to affect feeding behaviour and intake. With improved aerobic stability and reduction of yeasts and moulds, inoculation improved milk quality, reflected in lowered somatic cell count (SCC) in dairy cows fed BST silage. Conversely, SCC in cows fed spontaneously fermented silage were rising throughout the feeding period. A direct effect between diet and udder health is generally not given. Supporting udder health over the offered diet is aiming to increase feed quality, especially feed hygiene and amount of toxin contamination (Spiekers et al., 2009). It can be concluded that improved feed hygiene in the BST silage might increase udder health reflected in reduced SCC.

NEL density of the BST silage was higher compared to the CTL one, and it can be assumed that higher energy density of silage supported dairy cow performance. In addition, the BST silage contained higher amounts of lactate positively affecting performance. The main end product of the lactate fermentation in the rumen is propionate, a substrate for hepatic gluconeogenesis and lactate synthesis in the udder (Huhtanen et al., 2013). Recently discussed in literature was the positive influence of propane-1,2-diol, produced by heterofermentative bacteria (*L. brevis* and *L. kefiri*) and further conversion to propane-1-diol and propionate (Daniel et al., 2018). Results have been inconclusive; however, a study done by Lau et al. (2018) showed that the produced amount (0.389 g kg^{-1} DM) of propane-1,2-diol in grass silage increased milk yield and positively affected energy balance of dairy cows. These results demonstrate that secondary fermentation of lactic acid in silage may have a higher impact on animal performance than considered today.

Besides lactate as pre-cursor, higher propionate levels in combination with lowered ammonia nitrogen in the BST silage could positively enhance rumen nitrogen to energy balance resulting in better nutrient utilization and performance. As described in literature (Huhtanen et al., 2003), a reduction of 10 g kg^{-1} NH_3-N would be expected to increase ECM by 0.19 kg day^{-1}. Overall, ECM did not differ between the treatments, but yield of cows fed BST silage improved over time with significant differences starting from day 62 until end of the experiment, paralleled by a decline of cows fed CTL one.

Further research is needed to investigate, why effects did not occur within an expected adaptation period, but changes in milk yield were seen with a slow response and prolonged time of application. In contrast to decreased amounts of fibre components and only numerically increased acetate concentration in the BST silage, milk fat percentage was increased suggesting that increased cell wall degradation was leading to increased fibre fermentation in the rumen and, therefore, higher production of acetate. Weinberg et al. (2004) suggested that some LAB have a positive probiotic effect and that they interact with rumen microorganisms to such a way that rumen fibre degradability is enhanced.

During ensiling, bacterial fermentation is leading to proteolysis of the hydrophobic protein matrix surrounding the starch granules, which can be affected by LAB and may increase starch digestibility of silage (Silva da et al., 2017). Taken together, these findings suggest that beyond improved fermentation profile and silage quality, inoculation of silage improved nutrient availability and utilization as well as fermentation in the rumen itself. This theory is also supported by higher feed conversion ratio for cows fed BST silage compared to the CTL one. Muck et al. (2007) tested inoculants *in vitro* and, surprisingly, described increased microbial biomass production and reduced acetate to propionate ratios; therefore, it was speculated if inoculants alter microbial fermentation directly.

Conclusions

1. Inoculation of whole-plant maize forage with homo- and heterofermentative lactic acid bacteria (LAB) inoculant containing *Lactobacillus plantarum*, *L. brevis* and *L. kefiri* and applied at a rate of 2×10^5 cfu g^{-1} resulted in the improved fermentation profile of the silage by increasing the proportions of lactic acid and acetic acid and reducing the concentrations of undesirable butyric acid, alcohols and ammonia nitrogen (NH_3-N).

2. The inoculant treated (BST) silage was found to be more resistant to aerobic deterioration due to the restricted growth of yeast and mould. This was particularly evident in the assessment of the lower temperature increase of the aerated silage and improved aerobic stability by 2.6 days.

3. Inoculant treatment reduced fresh and dry matter loss and increased energy density of the silage resulting in increased metabolizable energy and netto energy lactation content.

4. A comparison of silage feeding value revealed significantly improved feed efficiency and significantly lowered somatic cell count in dairy cows fed BST silage, but no significant effect on milk yield throughout the trial period was observed. However, due to increased milk fat content cows fed BST silage had significantly higher fat and energy corrected milk yield from day 69 until end of the experiment.

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Gyvybingos pieno rūgšties bakterijos kaip priedas kukurūzų silosui: poveikis siloso fermentacijai, aerobiniam stabilumui ir juo šertų melžiamų karvių produktyvumui

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Santrauka

Kukurūzų vegetacinės masės, turinčios 355 g kg^{-1} sausųjų medžiagų (SM), silosas buvo pagamintas 200 tonų talpos gelžbetoninėse tranšėjose pridedant gyvų pieno rūgšties bakterijų inokulianto BioStabil (BST), sudaryto iš *Lactobacillus plantarum*, *L. brevis* ir *L. kefiri* padermių pagal 2×10^5 kvs g^{-1} silosuojamos masės normą arba be jokių priedų (kontrolinis variantas, CTL). Tranšėjos buvo atidarytos nuo silosavimo pradžios praėjus 92 dienoms, paimti mėginiai maisto medžiagų sudėčiai, fermentacijos rodikliams, aerobiniam stabilumui bei mikrobų kolonijų skaičiui nustatyti ir pradėtas šėrimo eksperimentas su melžiamomis karvėmis. Aerobiniam stabilumui nustatyti silosas buvo veikiamas oro. Šėrimo eksperimentas buvo vykdytas su 36 melžiamomis karvėmis, esančiomis 120-oje laktacijos dienoje, suskirstytomis į dvi analogines grupes: vienos grupės karvės buvo šertos neinokuliuotu (CTL), kitos – BST silosu, abiem grupėms pridėjus vienodą kiekį kombinuoto pašaro. Inokulianto priedas sumažino siloso rūgštumo (pH) vertę, padidino pieno ir propiono rūgščių koncentracijas ir sumažino amoniakinio azoto, etanolio bei sviesto rūgšties koncentracijas. Silose su BST inokulianto priedu sumažėjo užterštumas mielėmis ir pelėsiais, dėl to padidėjo jo aerobinis stabilumas, palyginus su silosu be priedų (CTL). Inokuliantas padidino energijos koncentraciją silose ir pagal riebalų bei energijos rodiklius koreguoto pieno kiekį nuo 69 šėrimo dienos iki bandymo pabaigos (92 dienos). BST silosu šertų karvių piene buvo didesnis riebalų procentas, panašus kiekis baltymų ir mažesnis kiekis somatinių ląstelių, palyginus su karvėmis, šertomis neapdorotu silosu (CTL). Pašaro konversija ir efektyvumas buvo geresnis karvių, šertų BST silosu. Su homo- ir heterofermentinių pieno rūgšties bakterijų priedu pagamintas silosas pakeitė fermentacijos profilį, padidino energijos koncentraciją, pagerino pašaro higieną bei aerobinį stabilumą, maisto medžiagų konversiją ir pieno sudėtį.

Reikšminiai žodžiai: aerobinis stabilumas, fermentacija, inokuliantas, tranšėja, pašaro efektyvumas.