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## THE EFFECT OF HOMO - AND HETERO - FERMENTATIVE LACTIC ACID BACTERIA MIX ON THE ENSILED LUCERNE FERMENTATION CHARACTERISTICS AND AEROBIC STABILITY IN BIG BALES

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The purpose of this study was to determine the effect of homo- and hetero-fermentative lactic acid bacteria mix on the ensiled lucerne fermentation characteristics and aerobic stability in big bales. The lucerne was ensiled without additives (C) and treated with a mix of bacterial inoculant that contains *Lactococcus lactis* and *Lactobacillus buchneri* (50:50) (I). Silage was treated with bacterial inoculant, which significantly increased the total organic acids concentration by 69 %, lactic acid by 92% and acetic acid by 76 %. If the results were compared with the C silage, the inoculation significantly decreased the concentrations of butyric acid by 73 %, ethanol by 53 % and ammonia - N concentration by 33%. Inoculated silage had significantly lowered the yeast count by 59 % and moulds count by 34 %. Compared to the inoculated silage and during the aerobic exposure, the untreated silage maximum temperature was significantly higher (13.9 °C vs 4.6 °C) (P < 0.05). Therefore, the bacterial inoculant improved the quality of fermentation and aerobic stability in lucerne silages.

*Keywords: aerobic stability, lactic acid bacteria, lucerne, silage.*

### INTRODUCTION

Silage is one of the most important feed for cattles, and it is usually make up the largest part of the diet. The quality of the silage is one of the main factors that govern the health of cows and good quality products. (Baliukonienė et al., 2012). The available scientific data suggests that the quality of the silage is generally poor and it is, therefore, necessary to pay more attention to the quality of the feed.

The quality of the silages are affected by many factors, for example equipment and additives used during the ensiling process. The composition of the forage at ensiling as well as technique of silage preparation is important (Wyss and Latsch, 2015). It is known that monitoring of the silage making process can be a key factor in the silage fermentation, but even when controlling the process, the silage quality can vary greatly (Kasmaei et al., 2013).

After opening of the silo, yeast and moulds potentially can increase pH value and temperature of the silage as well as reduction of sugars. Loss of carbon dioxide and temperature increase cause dry matter losses and reduce the feeding value of silage (Muck, 2012). The homofermentative lactic acid bacteria (LAB) produces lactic acid and are most commonly used to preserve silage during storage, meanwhile, heterofermentative LAB produces lactic acid, CO<sub>2</sub>, ethanol as well as acetic acid which inhibits the growth of yeast and moulds (Milora et al., 2015).

Recently, inoculants containing homo- and hetero-fermentative LAB has become predominant additives, because the combination of both types of these LAB can reduce losses and increase the fermentation quality as well as aerobic stability of silage (Li et al., 2016). However, there is still a lack of information on the effects of *Lactobacillus buchneri* in combination with the *Lactococcus lactis* on the ensiled lucerne fermentation and the aerobic stability in big bales.

The purpose of this study was to determine the effect of homo- and hetero-fermentative lactic acid bacteria mix on the ensiled lucerne fermentation characteristics and aerobic stability in big bales.

### MATERIALS AND METHODS

The experiment was held in 2016 at the Institute of Animal Science of Lithuanian University of Health Science. All materials and methods were made according to the DLG (Deutsche Landwirtschafts-Gesellschaft e. V. /internationally acknowledged German Agricultural Society), guidelines for the testing silage additives.

The silages were made from the severely ensiled second growth cut lucerne that was wilted for 7-8 h. The Lucerne was harvested from one field and was baled into the 1.2 m wide and 1.2 m length cylindrical bales as well as wrapped with 6 layers of white coloured stretch pellicle (the width of 750 mm and the thickness of 0.025 mm). We have prepared twenty big bales in total, ten with a mix of bacterial inoculant containing *Lactococcus lactis* and *Lactobacillus buchneri* (50:50) (I) and ten without any additives (C). The amount of the inoculant, that was applied to the herbage, was 150 000 cfu g<sup>-1</sup> also adding 4 L suspension for 1 tonne of lucerne. Moreover the same amount (4 L/t) of water was used instead of the suspension in the control treatment for the spontaneous fermentation. The blend of the bacterial strains was sprayed during the harvesting process while using the commercial sprayer HP-20. Samples of the water used for inoculation were collected and analyzed immediately (within an hour), using the ISO 15214 method. Both silages were kept in big bales for 120 days at outdoor temperature. Average of ambient temperature was 2.9°C. Five representative samples (>500 g each) of harvested herbage were taken before the ensiling period. At the sampling time of silages on day 120 of the ensiling period, five replications per treatment (10 big bales) were weighed for determination of DM loss and subsequently opened and sampled to analyze the DM content, pH, fermentation products and ammonia - N. The remaining 10 big bales (5 from each treatment) were used for the aerobic stability outdoor measurement. Aerobic stability was measured using data loggers that were placed in 5 controlling big bales and in 5 inoculated big bales to monitor temperature change inside the silage automatically every 6 hours, for up to 30 days (from day 120 to day 150 of storage). The aerobic deterioration was denoted by hours until the start of the sustained increase in temperature by more than 3°C above the ambient temperature.

Statistical analysis was determined using the SPSS program 20.0 for Windows. The results are presented as mean and standard deviation (mean±SD) and 95% confidence interval for mean. The results were considered to be reliable under P ≤ 0.05.

## RESULTS

The chemical and microbiological composition of fresh lucerne is summarized in Table 1. The lucerne was wilted up to 328 g kg<sup>-1</sup> and had low WSC (50.1 g kg<sup>-1</sup> DM) concentration while the concentrations of crude protein (220.2 g kg<sup>-1</sup> DM) and buffering capacity (48.9 mEq 100 g DM<sup>-1</sup>) were high. Consequently, lucerne was difficult to ferment.

Table 1. Chemical and microbiological composition of a fresh lucerne at ensiling

Variable	n	mean	SD
Dry matter g kg <sup>-1</sup>	5	327.6	4.74
Crude protein g kg <sup>-1</sup> DM	5	220.2	8.53
Crude fat g kg <sup>-1</sup> DM	5	18.8	2.39
Crude fibre g kg <sup>-1</sup> DM	5	293.6	19.45
Crude ash g kg <sup>-1</sup> DM	5	75.2	2.20
WSC g kg <sup>-1</sup> DM	5	50.14	2.11
NDF g kg <sup>-1</sup> DM	5	431.2	6.35
ADF g kg <sup>-1</sup> DM	5	331.1	7.76
Yeast log <sub>10</sub> cfu g <sup>-1</sup>	5	4.83	0.52
Moulds log <sub>10</sub> cfu g <sup>-1</sup>	5	4.65	0.33
LAB log <sub>10</sub> cfu g <sup>-1</sup>	5	4.92	0.07
Buffer capacity mEq 100 g DM <sup>-1</sup>	5	48.9	6.63
pH	5	6.17	0.02

Note. DM – dry matter, WSC – water-soluble carbohydrate, ADF – acid detergent fibre, NDF – neutral detergent fibre, LAB – lactic acid bacteria, cfu – colony forming units, SD – standart deviation.

The results of chemical and microbiological analyses of the silages after 120 days of ensiling are submitted in Table 2. Application of the bacterial inoculant resulted in a higher dry matter content by 3.1 % (P < 0.001) and less crude fiber content by 4.4 % (P < 0.01). We have noticed that the inoculant treatment reduced DM losses by 44 % (P < 0.001). Compared to the untreated silage, the inoculant treatment significantly increased the total organic acids concentration by 69 % (P < 0.001), more lactic acid by 92 % (P < 0.001), as well as much higher levels of acetic acid by 76% (P < 0.001). Compared to C, the inoculation significantly decreased the concentrations of undesirable fermentation products such as butyric acid by 73% (P < 0.001) and ethanol by 53 % (P < 0.001). Compared to the C, the treatment with the bacterial mix reduced proteolysis of plant proteins, because ammonia – N concentration was lowered by 33 % (P < 0.001) in the inoculated silage. The inoculant treatment increased the fermentation rate with a significantly deeper pH value by 0.34 units (P < 0.001). Inoculated silage had significantly (P < 0.001) lower yeast (1.18 vs 2.88) and moulds (1.42 vs 2.15) count and higher LAB count (7.44 vs 6.12) (P < 0.001) compared to control silage.

The results of aerobic stability measurements of the silages are presented in Figure 1. Inoculated silage had significantly lower sum of mean temperature (209.0 °C vs 390.4 °C) (P < 0.05) as well as maximum sample temperature compared to control silage (4.6 °C vs 13.9 °C) (P < 0.05).

Table 2. Chemical and microbiological composition of the silages after 120 days of storage

Variable	n	C	I
Dry matter, corrected for volatiles g kg <sup>-1</sup> Lower Bound – Upper Bound Mean ± SD	5	308.14 – 316.26 312.22±3.29	320.04 – 323.96 321.94±1.72**
DM losses g kg <sup>-1</sup> DM Lower Bound – Upper Bound Mean ± SD	5	92,41 - 100,53 96.5±4.62	50,25 - 58,38 54.3±3.11**
Crude protein g kg <sup>-1</sup> DM Lower Bound – Upper Bound Mean ± SD	5	209,69 - 216,53 213.1±2.25	213,89 - 220,73 217.3±4.11
Crude fibre g kg <sup>-1</sup> DM Lower Bound – Upper Bound Mean ± SD	5	304.42 – 315.18 309.8±5.71	290.88 – 301.64 296.3±4.67*
WSC g kg <sup>-1</sup> DM Lower Bound – Upper Bound Mean ± SD	5	5.79 – 9.72 7.75±2.52	5.79 – 9.73 7.76±1.18
NDF g kg <sup>-1</sup> DM Lower Bound – Upper Bound Mean ± SD	5	445.57 – 471.4 458.5±17.91	440.12 – 465.94 453.0±12.87
ADF g kg <sup>-1</sup> DM Lower Bound – Upper Bound Mean ± SD	5	362.29 – 386.73 374.5±15.78	356.2 – 380.65 368.4±12.77
Total acids g kg <sup>-1</sup> DM Lower Bound – Upper Bound Mean ± SD	5	49,36 – 59,04 54.2 ± 3.90	84,43 – 99,17 91.8 ± 5.93**
Lactic acid g kg <sup>-1</sup> DM Lower Bound – Upper Bound Mean ± SD	5	27.19 – 36.81 32.0 ± 3.87	55.47 – 67.33 61.4 ± 4.78**
Acetic acid g kg <sup>-1</sup> DM Lower Bound – Upper Bound Mean ± SD	5	14.18 – 17.42 15.8 ± 1.30	26.18 – 29.42 27.8 ± 1.30**
Butyric acid g kg <sup>-1</sup> DM Lower Bound – Upper Bound Mean ± SD	5	4.89 – 6.07 5.49 ± 0.68	0.91 – 2.09 1.50 ± 0.43**
Propionic acid g kg <sup>-1</sup> DM Lower Bound – Upper Bound Mean ± SD	5	0.47 – 0.88 0.67 ± 0.21	0.75 – 1.16 0.96 ± 0.19
Ethanol g kg <sup>-1</sup> DM 95% Confidence Interval for mean Lower Bound – Upper Bound Mean ± SD	5	6.76 – 9.24 8.0 ± 1.0	3.16 – 5.24 4.2 ± 0.84**
Ammonia N g kg <sup>-1</sup> total N Lower Bound – Upper Bound Mean ± SD	5	39.2 – 45.63 42.4 ± 3.87	25.41 – 31.83 28.6 ± 2.11**
Yeast log <sub>10</sub> cfu g <sup>-1</sup> Lower Bound – Upper Bound Mean ± SD	5	2.55 – 3.22 2.88±0.12	0.85 – 1.52 1.18±0.27**
Moulds log <sub>10</sub> cfu g <sup>-1</sup> Lower Bound – Upper Bound Mean ± SD	5	1.93 – 2.38 2.15±0.15	1.19 – 1.65 1.42±0.16**
LAB log <sub>10</sub> cfu g <sup>-1</sup> Lower Bound – Upper Bound Mean ± SD	5	5.91 – 6.32 6.12±0.18	7.23 – 7.64 7.44±0.31**
pH Lower Bound – Upper Bound Mean ± SD	5	4.75 – 4.83 4.79 ± 0.06	4.4 – 4.49 4.45 ± 0.05**

Note. DM – dry matter, WSC – water-soluble carbohydrate, ADF – acid detergent fibre, NDF – neutral detergent fibre, cfu – colony forming units, SD – standart deviation.

\* and \*\* denote statistically significant difference vs control at level 0.01 and 0.001, respectively.

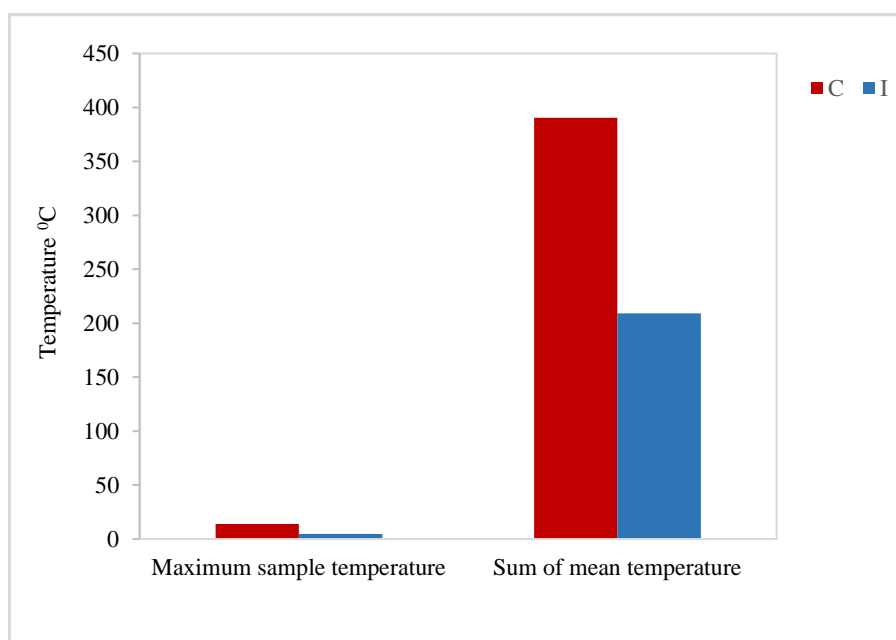


Figure 1. Aerobic stability measurements

## DISCUSSION

Our findings showing that LAB inoculation of lucerne improved silage fermentation by increasing lactic acid and reducing pH value. The obtained results meet the results provided by Hindrichsen et al., (2012), where *L. lactis* was superior in removing oxygen, pH reduction and clostridia inhibition. The lower ammonia - N concentration in the inoculated silage suggests that the inoculant reduced pectinolytic activity. These results confirm the researches received earlier by Vrotniakienė and Jatkauskas, 2014. The inoculant has substantially reduced the levels of unwanted fermentation products, for example ethanol and butyric acid concentrations. The data obtained by the latter, confirm the data of other researchers in which the heterofermentative LAB was used alone or together with homofermentative LAB (Schmidt and Kung, 2010).

We have noticed that the addition of bacterial inoculant resulted higher level of acetic acid. It is well known that *L. buchneri* produce acetic acid, which inhibits growth of yeast and moulds and as the result can increase aerobic stability of silages (Jatkauskas and Vrotniakienė, 2012). The present study demonstrated that the inoculated silage had significantly lower yeast and moulds count compared to the control silage. The addition of hetero + homo LAB effectively inhibited the growth of yeast in the silage (Li et al., 2016).

The use of silage inoculants based on hetero- and homo- fermentative LAB, has been used relatively recently, but according to recent researches, these bacteria have a positive effect on aerobic stability of silages (Kung et al., 2003; Kang et al., 2009; Wambacq et al., 2013).

## CONCLUSIONS

Addition of the bacterial inoculant containing of *Lactococcus lactis* and *Lactobacillus buchneri* in lucerne:

- a) gave the better fermentation, lower pH and accelerates the lactic acid and acetic acid formation;
- b) decreased of the concentration of undesirable fermentation products such as butyric acid and ethanol and significantly reduced the DM loss;
- c) reduced ammonia – N concentration and was effective in limiting the degradation of protein;
- d) improved the hygienic quality of the lucerne silage by lowering growth of moulds, which are able to produce potent mycotoxins that are harmful to animals and humans, and enhances silage aerobic stability.

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