**Original Paper**

# The effect of biological additive on nutrient composition of grass silages

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The aim of the study was to analyze the effect of biological additive on nutrient composition of grass silages. The experiment was realized in practical conditions on the farm RD Klenovec in 2013. Grass mass of permanent grassland was ensiled in silage bags. Laboratory analysis of silage samples were carried out at 8 weeks of fermentation, 6 and 12 months of storage. Into grass silage of experimental group was added biological additive, consisted of mixture of homofermentative and heterofermentative lactic acid bacteria. Inoculant included strains *Lactobacillus brevis* and *Lactobacillus plantarum*. There were not found significant differences ($P > 0.05$) between the control and experimental group in contents of nutrients in individual analysis of silage samples. We found higher values of dry matter, crude protein and organic matter, and lower content of ash after supplementation of biological additive in all analyzed samples. Tendency of ($P > 0.05$) a higher content of crude fiber was found after 8 weeks, 6 months of storage and fat after 12 months of storage. There were found significant ($P < 0.05$) differences in contents of NEL after 8 weeks and 12 months of storage and NEG only after 12 months of storage between control and experimental samples of silage.

**Keywords:** grass silage, fermentation, inoculant, nutrients

## 1. Introduction

Silage additives are used to improve the fermentation (Lád et al., 2006). Additives are also used for reduction of dry matter losses and preservation of nutrients during fermentation (Jaster, 1994). The reasons for which the additives apply into silage are inhibition of growth of aerobic microorganisms (*Listeria monocytogenes*), undesirable anaerobic microorganisms (*enterobacteria*, *clostridia*), inhibition of activity of plant and microbial proteases and deaminases, improving the stocks of fermentable substrates for lactic acid bacteria, the addition of beneficial microorganisms, improvement ensilability, nutritional value, utilization of nutrients and are nutrient source (Jaster, 1994; Buxton et al., 2003). Inoculant strain should be able to promote a rapid decline in the pH, survive throughout the fermentation process and improve the aerobic stability (Saarisalo et al., 2007). Homofermentative lactic acid bacteria (LAB) have been selected to increase lactic acid concentration in the silo, but aerobic stability may be impaired because lactic acid can be easily oxidized by yeasts when the silage is exposed to air (Pahlow et al., 2003). Heterofermentative LAB have attracted attention as an alternative additive to inhibit aerobic deterioration (Driehuis et al., 1999; Avila et al., 2009).

The aim of this study was to determine the effect of bacterial inoculant on changes in nutrient composition of grass silage.

## 2. Material and methods

The experiment was realized in practical conditions on the farm RD Klenovec in 2013. RD Klenovec is located in undermountain production area with considerable slope, altitude is 300–450 m.s.l. Grass mass of permanent grassland was ensiled in silage bags. Length of ensiled grass mass was 50 mm. Weight of cubic meter of silage was 550 kg in bag. Laboratory analysis of silage samples was carried out at 8 weeks of fermentation, 6 and 12 months of storage. The number of samples was three from each sampling. Into grass silage in experimental group was added biological additive, consisted of mixture of homofermentative and heterofermentative lactic acid bacteria, before opening the silage bags. Additive included strains *Lactobacillus brevis* and *Lactobacillus plantarum*. Inoculant contained $2 \times 10^5$ CFU per 1 gram. Dose of preservative was 4 g $t^{-1}$ of silage. At first was dissolved in water and then applied by using applicator Ziegler (type-FDG). Chemical analysis was conducted at the Laboratory of quality and nutritional value of feeds (Excelent Center for Agrobiodiversity Conservation and

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Benefit) at the Department of Animal Nutrition (Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra). After collecting complete analyze results of experiment, it was assessed the impact of inoculant on process of fermentation and changing nutrients in silage. Differences between groups were analyzed with one-way analysis of variance (ANOVA) by using the statistical programme SPSS 20.0. Results were evaluated by using Tukey test. Values with different superscripts within a column are significant at $P$ <0.05.

## 3. Results and discussion

Results of nutrients composition of grass mass and silage are shown in Table 1 and 2. We found significant ($P$ <0.05) differences in content of dry matter (DM) and fat in grass mass before ensiling. There were not found significant differences ($P$ >0.05) between the control and experimental group in individual analysis of samples, only among the all analyzes together in content of fat of grass silage. After supplementation of biological additive we found higher content of dry matter, crude protein and organic matter in all analyzes of grass silage samples. The optimal content of dry matter in grass silages should be 30–40% (Doležal et al., 2012). Guo et al. (2013) compared grass silages with 31% dry matter without and with the addition of *L. plantarum* + *L. buchneri* but they did not observe statistically significant differences in dry matter and crude protein content. The optimal mean concentration of crude protein in grass silage is approximately 160 g kg$^{-1}$ DM, although it can range from 39 to 282 g kg$^{-1}$ DM (Merry et al., 2000). Negative impact of inoculant was found in content of ash in all our analyzed samples. Tendency of ($P$ >0.05) a higher amount of crude fiber was found after 8 weeks, 6 months of storage and fat after 12 months of storage. Jalč et al. (2009) found higher content of dry matter (from 222. 8 g to 246.6 g kg$^{-1}$), crude protein (from 126.4 g to 139.9 g kg$^{-1}$ DM), lower content of ash (from 78.1 g to 75.9 g kg$^{-1}$ DM), fiber (from 409.5 g to 348.3 g kg$^{-1}$ DM), fat (from 24.9 g to 24.5 g kg$^{-1}$ DM) after addition *Lactobacillus plantarum*. Winters et al. (2001) reported positive effect of bacterial inoculants on content of DM and crude protein.

Results of nutritional value of grass mass and silage are shown in Table 3 and Table 4. In grass mass before ensiling

**Table 1** Content of nutrients in grass mass before ensiling

| Sample of grass mass | Statistical parameter | DM | CP | CF | Ash | Fat | OM |
|---|---|---|---|---|---|---|---|
| | | % | % of DM | | | | |
| Control | mean | 38.01$^{a}$ | 12.24 | 32.27 | 9.6 | 2.45$^{a}$ | 90.38 |
| | S.D. | 1.41 | 0.33 | 0.25 | 0.83 | 0.05 | 0.81 |
| Experiment | mean | 41.24$^{b}$ | 12.11 | 32.11 | 8.84 | 2.01$^{b}$ | 91.07 |
| | S.D. | 1.87 | 0.2 | 0.12 | 0.55 | 0.04 | 0.48 |

DM – dry mater, CP – crude protein, CF – crude fiber, OM – organic matter, S.D. – standard deviation

**Table 2** Content of nutrients in grass silage

| Analysis of samples | Sample of silage | Statistical parameter | DM | CP | CF | Ash | Fat | OM |
|---|---|---|---|---|---|---|---|---|
| | | | % | % of DM | | | | |
| 8 weeks | control | mean | 35.65 | 12.32 | 31.27 | 9.52 | 2.26$^{a}$ | 90.48 |
| | | S.D. | 0.73 | 0.02 | 1.23 | 0.1 | 0.08 | 0.1 |
| | experiment | mean | 37.02 | 12.18 | 32.16 | 9.14 | 2.26$^{a}$ | 90.86 |
| | | S.D. | 0.19 | 0.18 | 0.52 | 0.04 | 0.07 | 0.04 |
| 6 months | control | mean | 35.22 | 12.44 | 31.67 | 9.87 | 2.34$^{ab}$ | 90.13 |
| | | S.D. | 0.9 | 0.26 | 0.16 | 0.03 | 0.05 | 0.03 |
| | experiment | mean | 36.55 | 12.54 | 31.72 | 9.16 | 2.32$^{ab}$ | 90.84 |
| | | S.D. | 0.57 | 0.29 | 17.14 | 0.07 | 0.04 | 0.07 |
| 12 months | Control | Mean | 35.79 | 12.44 | 32.16 | 9.68 | 2.37$^{ab}$ | 90.32 |
| | | S.D. | 0.38 | 0.37 | 0.1 | 0.06 | 0.05 | 0.06 |
| | Experiment | Mean | 36.85 | 12.83 | 31.47 | 9.17 | 2.43$^{b}$ | 90.83 |
| | | S.D. | 0.21 | 0.1 | 0.07 | 0.08 | 0.03 | 0.08 |

DM – dry mater, CP – crude protein, CF – crude fiber, OM – organic matter, S.D. – standard deviation



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**Table 3** The nutritional value of the grass mass before ensiling

| Sample of grass mass | Statistical parameter | NEL | NEG | PDIN | PDIE |
|---|---|---|---|---|---|
| | | MJ kg$^{-1}$ of DM | g kg$^{-1}$ of DM | | |
| **Control** | mean | 5.2 | 4.88$^{a}$ | 73.9 | 75.04 |
| | S.D. | 0.02 | 0.1 | 0.63 | 1.56 |
| **Experiment** | mean | 5.23 | 5.02$^{b}$ | 75.1 | 74.3 |
| | S.D. | 0.03 | 0.01 | 0.85 | 0.47 |

NEL – net energy for lactation, NEG – net energy for gain, PDI – true protein digested in the small intestine, S.D. – standard deviation

**Table 4** The nutritional value of the grass silage

| Analysis of samples | Sample of silage | Statistical parameter | NEL | NEG | PDIN | PDIE |
|---|---|---|---|---|---|---|
| | | | MJ kg$^{-1}$ of DM | g kg$^{-1}$ of DM | | |
| **8 weeks** | control | mean | 5.22$^{a}$ | 5.13$^{a}$ | 74.1 | 70.59 |
| | | S.D. | 0.03 | 0.03 | 0.26 | 2.25 |
| | experiment | mean | 5.33$^{b}$ | 5.14$^{a}$ | 72.51 | 71.56 |
| | | S.D. | 0.03 | 0.02 | 1.14 | 1.03 |
| **6 months** | control | mean | 5.28$^{ab}$ | 5.22$^{ab}$ | 73.97 | 70.34 |
| | | S.D. | 0.01 | 0.02 | 0.17 | 3.16 |
| | experiment | mean | 5.35$^{bc}$ | 5.26$^{b}$ | 72.83 | 71.62 |
| | | S.D. | 0.03 | 0.03 | 0.56 | 2.17 |
| **12 months** | control | mean | 5.31$^{b}$ | 5.19$^{ab}$ | 73.77 | 69.61 |
| | | S.D. | 0.01 | 0.01 | 0.2 | 3.08 |
| | experiment | mean | 5.4c | 5.3$^{c}$ | 73.4 | 71.95 |
| | | S.D. | 0.02 | 0.02 | 0.49 | 1.93 |

NEL – net energy for lactation, NEG –: net energy for gain, PDI: true protein digested in the small intestine, S.D.: standard deviation

we found statistically significant ($P$ <0.05) differences only in content of NEG. Higher contents were found in NEL, NEG, PDIN and in PDIE was found lower content after addition of biological additive before ensiling. There were observed significant ($P$ <0.05) differences in contents of *NEL* after 8 weeks and 12 months of storage and NEG only after 12 months of storage between control and experimental samples of silage. Tendency

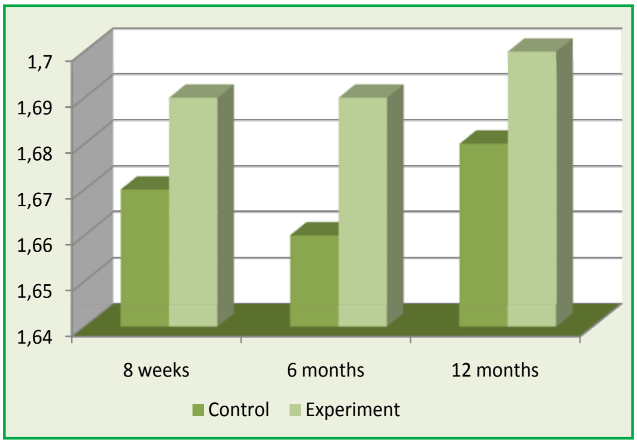


**Figure 1** Production efficiency of grass silage

of ($P$ >0.05) a higher content of NEL, NEG and PDIE was found in inoculated silage. Jančová (2009) and Zurek and Wróbel (2006) found similar values of NEL, NEG, PDIN and PDIE. Doležal and Hejduk (2002) also reported higher value *NEL* in inoculated silage. There were found contents of NEL 6.1 and 6 MJ kg$^{-1}$ of DM in grass-white clover and grass-red clover silages (Steinshamn and Thuen, 2008). Jančová (2014) observed in grass silage with dry matter content 361.07 g kg$^{-1}$ value NEL 5.52 MJ kg$^{-1}$, NEG 5.37 MJ kg$^{-1}$ of DM, PDIN 71.49 g kg$^{-1}$ of DM and PDIE 69.64 g kg$^{-1}$ of DM. *NEL* ranged 4.6–6.61 MJ kg$^{-1}$ of DM and NEG 5.06–9 MJ kg$^{-1}$ of DM (Aston et al., 1995). In the study Burke et al. (2007), were found contents of PDIN 104 g kg$^{-1}$ of DM and PDIE 75 g kg$^{-1}$ of DM in grass silage. Winters et al. (2001) reported positive effect of bacterial inoculant on values of NEL and NEG.

Results of production efficiency of grass silage are shown in Figure 1. We did not found significant ($P$ >0.05) differences between control and experimental group. The highest production efficiency was observed after 12 months of storage. Jančová (2014) reported production efficiency of grass silage 1.74 kg. Zurek and Wróbel (2006) presented similar production efficiency. There was found



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1.92 kg production efficiency (Steinshamn and Thuen, 2008).

## 4. Conclusions

Biological additive (*Lactobacillus brevis* and *Lactobacillus plantarum*) lead to an increase of dry matter, crude protein and organic matter in all analysed of grass silage samples. Content of ash decreased in experimental samples of silage. Differences between control and experimental group were not statistically significant ($P > 0.05$). Tendency of ($P > 0.05$) a higher amount of crude fiber was found after 8 weeks, 6 months and fat after 12 months of storage. There were found significant ($P < 0.05$) differences in contents of *NEL* after 8 weeks and 12 months of storage and NEG only after 12 months of storage between control and experimental samples of silage. Tendency of ($P > 0.05$) a higher contents of *NEL*, NEG and PDIE was found in inoculated silage. We did not found significant ($P > 0.05$) differences between control and experimental group in production efficiency of silage.

## 5. Acknowledgement

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