



# The effect of biological and chemical additives on the chemical composition and fermentation process of *Dactylis glomerata* silage

Jhonny E. Alba-Mejía<sup>1</sup>, Jiří Skládanka<sup>1</sup>, Alois Hilgert-Delgado<sup>2</sup>, Miroslav Klíma<sup>2</sup>, Pavel Knot<sup>1</sup>, Petr Doležal<sup>1</sup> and Pavel Horky<sup>1</sup>

<sup>1</sup> Mendel University in Brno, Faculty of AgriScience, Department of Animal Nutrition and Forage Production, 61300 Brno, Czech Republic

<sup>2</sup> Crop Research Institute, Division of Crop Genetics, 16106 Prague, Czech Republic

## Abstract

This study was carried out to determine the chemical composition, silage quality and ensilability of ten cocksfoot cultivars using biological and chemical silage additives. The plant material was harvested from the first and second cut, cultivated at the Research Station of Fodder Crops in Vatin, Czech Republic. Wilted forage was chopped and ensiled in mini-silos with 3 replicates per treatment. The treatments were: 1) without additives, used as a control; 2) with bacterial inoculants; and 3) with chemical preservatives. The results indicated that the year factor (2012-2013) influenced significantly the chemical composition of the silage in both cuts. The use of biological inoculants reduced the content of crude fibre and acid detergent fibre; but it did not influence the content of neutral detergent fibre, in comparison with the control silage in both cuts. Furthermore, the application of biological inoculants reduced the concentration of lactic acid (LA) and acetic acid (AA) in contrast to the control silage in the first cut. Moreover, in the second cut the same values tended to be the opposite. Interestingly, 'Amera' was the unique variety that presented a high concentration of butyric acid (0.2%) in comparison with other varieties in the first cut. In conclusion, the biological inoculants had a favourable effect on silage fermentation. Notably, only 'Greenly' and 'Starly' varieties from the first cut; and 'Greenly', 'Sw-Luxor', and 'Otello' varieties from the second cut were appropriate for ensiling because their pH-values; LA and AA concentrations were ideal according to the parameters of the fermentation process.

**Additional key words:** cocksfoot; ensiling; feeding value; forage preservation; grass varieties; mini-silo; fermentation quality.

**Abbreviations used:** AA (acetic acid); ADF (acid detergent fibre); AWE (acidity of water extract); BA (butyric acid); BSA (biological silage additives); CF (crude fibre); CFU (colony forming units); CSA (chemical silage additives); CP (crude protein); DM (dry matter); LA (lactic acid); NDF (neutral detergent fibre); OMD (organic matter digestibility); PA (propionic acid); SA (silage additives); SEM (standard error of the mean); US (untreated silage); VFA (volatile fatty acid); WSC (water soluble carbohydrates).

**Authors' contributions:** Conceived and designed the experiments: JEAM, JS and PD. Performed the experiments: JEAM, JS and PK. Analyzed the data: JEAM, JS, AHD and MK. Contributed reagents/materials/analysis tools: JEAM, PK and PH. Wrote the paper: JEAM, JS, AHD and MK.

**Citation:** Alba-Mejía, J. E.; Skládanka, J.; Hilgert-Delgado, A.; Klíma, M.; Knot, P.; Doležal, P.; Horky, P. (2016). The effect of biological and chemical additives on chemical composition and fermentation process of *Dactylis glomerata* silage. Spanish Journal of Agricultural Research, Volume 14, Issue 2, e0604. <http://dx.doi.org/10.5424/sjar/2016142-8040>.

**Received:** 20 May 2015. **Accepted:** 23 May 2016

**Copyright** © 2016 INIA. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial (by-nc) Spain 3.0 Licence, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Funding:** Czech National Agency for Agricultural Research (Grant No. QJ1310100 "Development and optimization methods for the determination of biogenic amines in response to increasing health security of silage"); Mendel University in Brno (IGA TP 2/2015 "Effect of selenium on the quality of plant and animal production from the perspective of safety").

**Competing interests:** The authors have declared that no competing interests exist.

**Correspondence** should be addressed to Jhonny E. Alba-Mejía: [jeam1604@gmail.com](mailto:jeam1604@gmail.com).

## Introduction

Cocksfoot (orchard grass, *Dactylis glomerata* L.) is a long-lived, perennial grass with excellent regrowth characteristics and adaptability to various environmental conditions (Sanada *et al.*, 2010). It is commonly recommended for pastures, owing to drought resistance and winter-hardiness (Sanderson *et al.*, 2002). *Dactylis*

*glomerata* L. is a versatile grass utilized for grazing, hay, or silage production on a global scale due to its high forage quality (*i.e.*, sugar and protein contents), shade tolerance, and persistence (Lindner *et al.*, 2004). This forage grass has a high economic value due to its high productivity and disease resistance under alternating weather conditions (Mika *et al.*, 2002). In view of

climate conditions, silage is the best method to preserve fresh forage material with minimal losses of nutritive value by fermentation of soluble carbohydrates in an anaerobic environment. During the ensiling procedure, silage quality and nutritional value are affected by numerous biological and technological factors (Sarıçiçek & Kiliç, 2009). Some of these are the crop species, stage of maturity and dry matter (DM), content at green forage, chop length, type of silo, rate of filling, forage density after packing, sealing technique, weather conditions at harvest, and additive use (Pozdíšek *et al.*, 2003).

It is evident that the additives based on lactic acid (LA) bacteria can significantly enhance the fermentation quality of silages (Filya *et al.*, 2007). In recent years, it was found that improvement of both the efficiency of the anaerobic fermentation and the aerobic stability of silage forage could be achieved through the use of several recently found types of dual purpose inoculants (Mohammadzadeh *et al.*, 2012). The spectrum of bacteria contains, on the one hand, homo-fermentative species merely producing lactic acid; and on the other hand, hetero-fermentative species producing a compound of lactic and acetic acids as well as other by-products (*e.g.*, ethanol and carbon dioxide, among others) (Vlková *et al.*, 2012). The varieties of homo- or hetero-fermentative inoculants are dependent on the aim of inoculation. Thus, while hetero-fermentative inoculants are more efficient in the maintenance of the aerobic stability of silages, homo-fermentative inoculants are stronger in the improvement of the fermentation features (Kung *et al.*, 2003).

Up to now, silage research has placed special emphasis on filling the gap between the feeding value of the original crop and that of the resulting silage (Charmley, 2001). For this reason, the assessment of silage quality can be considered as a management tool of high importance on the farm (Huhtanen *et al.*, 2007).

The objectives of this study were to determine the ensilability of ten varieties of *Dactylis glomerata* L., and to evaluate whether the treatment with bacterial inoculants and chemical additives would improve the quality of the fermentation process and/or the chemical composition of the silages.

## Material and methods

### Experimental site

A field experiment was conducted at the Research Station of Fodder Crops in Vatin, Czech Republic (49°31'N, 15°58'E), and established in 2011 at the altitude of 560 m.a.s.l. The climate conditions at the

station can be characterized by an annual average precipitation of 632 mm, 658 mm, 705 mm, and 819 mm; with a mean annual temperature of 7.4°C, 6.8°C, 7.3°C, and 8.7°C in the years 2011, 2012, 2013, and 2014, respectively. The soil type is a Cambisol as sandy-loam on a diluvium of biotic orthogenesis. A split-plot design was used with plots of 1.5 × 10 m. The plots were harvested using a self-propelled mowing machine (HEGE 212 harvester, Wintersteiger, Ried im Innkreis, Austria) with a mowing width of 1.25 m. The harvested area was 12.5 m<sup>2</sup>, and the remaining stubble height was 7 cm. The experiment was carried out with three replicates.

### Plant materials

Ten varieties of *Dactylis glomerata* L. from different countries were used in this investigation: 'Greenly' and 'Starly' from France; 'Sw-Luxor' from Sweden; 'Otello' from Italy; 'Husar' from Germany; 'Amera', 'Dika', and 'Bepro' from Poland; and 'Dana' and 'Vega' from the Czech Republic. Each variety was sown with 20 kg/ha of seeds. The trial was established on 14<sup>th</sup> April 2011, and the assessment took place in 2012 and 2013. The experimental plots were fertilized with 60 kg/ha N per year. The first and second growth were harvested according to the heading phase (vegetation stage when inflorescence is emerging, but before shedding pollen). The herbage was wilted on the plot for 14 hours in order to reduce the water content after mowing. Afterwards, the grass material was transported to the laboratory of the Department of Animal Nutrition and Forage Production, Faculty of Agronomy (Mendel University in Brno). Samples, each of 10 kg per treatment, were taken and chopped with a conventional forage harvester under laboratory conditions to a particle length of 40-60 mm.

### Treatment of grass materials

Representative forage samples (6 kg) were filled into mini-silos of polyvinyl chloride, and compacted with a pressure of 600 kg/m<sup>3</sup>. The filled silos (three repetitions per treatment) were sealed with a lid and stored in a room without direct light exposure at room temperature of 28°C for 90 days. The following treatments were applied to the forage samples: 1) silage without inoculants, used as control; 2) silage containing biological inoculants [*i.e.*, *Lactobacillus plantarum* (DSMZ 16568) 5 · 10<sup>10</sup> CFU/g, *Lactobacillus buchneri* (DSMZ 22501/CCM 1819, DSZM: German collection of microorganisms and cell cultures; CCM: Czech col-

lection of microorganisms)  $1.25 \cdot 10^{10}$  CFU/g, and *Enterococcus faecium* (DSMZ 22502/NCIMB, NCIMB: Scotland national collection of industrial and marine bacteria)  $6.25 \times 10^{10}$  CFU/g; and 3) silage containing chemical additives [*i.e.*, formic acid, propionic acid, benzoic acid, ammonium formate, E150d-sulfite ammonia, caramel (manufacturer BIOFERM CZ, spol. s r.o., Czech Republic), and water]. Biological inoculants were applied at a rate of 2 g/t, and chemical additives at 4 L/t, according to the official manufacturer's recommendation. At the end of the ensiling period (90 days) the silos were opened and samples were taken for the respective chemical analysis.

### Biomass chemical analysis

Dry matter was analysed after drying the biomass at 103°C. Meanwhile, the pre-drying of subsamples (green matter and silage) was performed in a specific drying oven at 60°C for 48 hours to determine the nutritive composition. Subsequently, the forage samples for analysis of nutritive value were ground in a mill and then filtered through a 1 mm sieve. The green matter and silage samples were analysed for dry matter (DM), crude protein (CP), crude fibre (CF), acid detergent fibre (ADF), neutral detergent fibre (NDF), and organic matter digestibility (OMD). The content of nutrients was determined according to ČSN 467092 standards (1997). The CP content was determined by using Kjeltac 2300 equipment (Foss, Denmark), and the CF content was determined by using the Fibre analyser (ANKOM 220, USA). As to individual param-

eters of interest, OMD was detected *in vitro* by the pepsin-cellulose method (Doležal, 2002).

Furthermore, forage samples were analysed for: pH-value; lactic acid (LA); volatile fatty acids (VFAs) such as acetic acid (AA), butyric acid (BA), and propionic acid (PA); and acidity of water extract (AWE) 90 days after ensiling (AOAC, 1980). The analytical procedures including the preparation of water extracts were described previously by Doležal (2002).

### Statistical analyses

The data were processed using the statistical software STATISTICA.CZ Version 12 (Prague, Czech Republic). The results are expressed as a mean  $\pm$  standard error of the mean (SEM). Differences with  $p < 0.05$  were considered significant and determined using multifactorial ANOVA, in particular, Scheffé's test, which was applied for comparing mean values. Cluster analysis was performed to create table representations.

## Results

### Chemical composition of green matter

The nutritive composition of the green forages from the first and second cut was determined in the experiments prior to ensiling (Tables 1 and 2). In terms of DM, CP, CF, ADF, NDF, and OMD values, the results did not show any significant differences – neither between varieties nor between the cuts.

**Table 1.** Chemical composition (%) of forage before ensiling - First cut 2012-2013.

Factor	DM	CP	CF	ADF	NDF	OMD
<b>Varieties (V)</b>						
Greenly	38.2 $\pm$ 1.7	8.3 $\pm$ 0.7	29.8 $\pm$ 3.6	32.7 $\pm$ 4.4	58.0 $\pm$ 5.8	85.9 $\pm$ 2.6
Starly	39.9 $\pm$ 2.2	8.1 $\pm$ 0.8	29.3 $\pm$ 3.7	31.8 $\pm$ 3.7	56.4 $\pm$ 5.8	88.2 $\pm$ 4.5
Sw-Luxor	39.6 $\pm$ 6.9	8.8 $\pm$ 0.6	29.2 $\pm$ 3.1	31.4 $\pm$ 3.1	56.2 $\pm$ 4.6	88.6 $\pm$ 3.1
Otello	39.7 $\pm$ 8.8	9.6 $\pm$ 0.3	28.7 $\pm$ 2.0	31.2 $\pm$ 1.6	56.1 $\pm$ 2.9	87.3 $\pm$ 5.7
Husar	34.3 $\pm$ 7.3	8.9 $\pm$ 0.2	29.4 $\pm$ 2.9	32.2 $\pm$ 3.3	56.5 $\pm$ 4.6	83.7 $\pm$ 4.9
Amera	30.8 $\pm$ 0.1	10.4 $\pm$ 0.0	29.5 $\pm$ 2.6	32.4 $\pm$ 2.7	56.9 $\pm$ 3.9	87.1 $\pm$ 2.2
Dika	39.8 $\pm$ 6.4	9.2 $\pm$ 0.2	28.6 $\pm$ 2.5	31.2 $\pm$ 1.6	55.4 $\pm$ 3.3	86.5 $\pm$ 3.1
Bepro	41.0 $\pm$ 9.2	8.6 $\pm$ 1.2	29.8 $\pm$ 3.2	32.7 $\pm$ 3.5	58.4 $\pm$ 4.4	86.9 $\pm$ 4.7
Dana	38.0 $\pm$ 6.7	9.1 $\pm$ 0.1	29.4 $\pm$ 1.9	32.2 $\pm$ 1.7	57.3 $\pm$ 2.2	85.1 $\pm$ 4.2
Vega	39.8 $\pm$ 6.0	8.7 $\pm$ 1.2	30.0 $\pm$ 4.1	32.9 $\pm$ 4.1	57.4 $\pm$ 6.0	87.5 $\pm$ 5.2
<b>Years (Y)</b>						
2012	42.8 $\pm$ 1.9 <sup>a</sup>	9.5 $\pm$ 0.2 <sup>a</sup>	26.4 $\pm$ 0.2 <sup>a</sup>	29.1 $\pm$ 0.2 <sup>a</sup>	52.5 $\pm$ 0.4 <sup>a</sup>	90.7 $\pm$ 0.6 <sup>a</sup>
2013	33.3 $\pm$ 1.4 <sup>b</sup>	8.5 $\pm$ 0.3 <sup>b</sup>	32.3 $\pm$ 0.3 <sup>b</sup>	35.0 $\pm$ 0.5 <sup>b</sup>	61.2 $\pm$ 0.6 <sup>b</sup>	82.7 $\pm$ 0.6 <sup>b</sup>

DM: dry matter; CP: crude protein; CF: crude fibre; ADF: acidic detergent fibre; NDF: neutral detergent fibre; OMD: organic matter digestibility. <sup>a,b</sup> superscripts within a row indicate statistical significance ( $p < 0.05$ ).

**Table 2.** Chemical composition (%) of forage before ensiling - Second cut 2012-2013.

Factor	DM	CP	CF	ADF	NDF	OMD
<b>Varieties (V)</b>						
Greenly	46.2 ± 1.5	9.0 ± 0.7	28.7 ± 0.0	33.7 ± 0.4	58.3 ± 0.0	79.1 ± 0.7
Starly	46.7 ± 2.7	8.5 ± 1.2	29.2 ± 0.4	34.5 ± 0.4	58.0 ± 0.3	80.5 ± 0.4
Sw-Luxor	46.9 ± 0.1	9.0 ± 0.3	30.2 ± 1.0	36.0 ± 0.9	60.5 ± 2.0	81.1 ± 1.9
Otello	50.1 ± 0.4	9.9 ± 0.1	29.4 ± 0.2	35.3 ± 0.1	59.3 ± 0.1	84.1 ± 3.9
Husar	50.2 ± 1.0	8.9 ± 0.2	28.6 ± 0.4	34.6 ± 0.2	57.3 ± 0.0	84.0 ± 4.1
Amera	52.6 ± 1.7	10.0 ± 0.2	29.1 ± 0.2	35.6 ± 0.1	57.3 ± 0.6	85.3 ± 4.6
Dika	48.4 ± 1.3	8.9 ± 0.7	30.0 ± 0.5	35.8 ± 0.1	58.7 ± 0.4	84.5 ± 2.6
Bepro	49.9 ± 3.1	9.5 ± 0.5	28.7 ± 0.6	34.5 ± 0.2	58.0 ± 0.3	83.2 ± 3.3
Dana	49.1 ± 0.3	9.1 ± 0.6	30.1 ± 0.7	35.6 ± 0.4	58.1 ± 0.4	83.7 ± 0.5
Vega	48.6 ± 1.6	9.0 ± 0.6	30.5 ± 0.9	36.0 ± 0.1	59.8 ± 0.2	83.8 ± 2.5
<b>Years (Y)</b>						
2012	48.8 ± 1.0	8.7 ± 0.3 <sup>a</sup>	29.9 ± 0.3 <sup>a</sup>	35.3 ± 0.3	58.8 ± 0.5	85.3 ± 1.1 <sup>a</sup>
2013	48.9 ± 0.5	9.6 ± 0.1 <sup>b</sup>	29.0 ± 0.2 <sup>b</sup>	35.0 ± 0.2	58.2 ± 0.2	80.5 ± 0.4 <sup>b</sup>

See legend of Table 1.

Concerning the first cut, the analysis demonstrated a higher content of DM, CP, and OMD; and a lower content of CF, ADF, and NDF in 2012 than in 2013 (Table 1). On the other hand, in the second cut, there was a lower content of CP, a higher content of CF and OMD, and no significant differences in the content of DM, ADF, and NDF in 2012, compared to the year 2013 (Table 2).

### Effects of additives on chemical composition of grass silage

After 90 days of ensiling, the chemical values of the tested cultivars were significantly affected by the treatment (Tables 3 and 4). The results showed that treated silages with biological inoculants and chemical additives had a significantly higher content of DM in contrast with the control in both cuts. In the first cut, the DM content was 36.1%, 36.8%, and 35.3%, respectively; and in the second cut, it was 47.2%, 47.4%, and 47.0%, respectively.

The contents of CF and ADF fractions for the variants treated with BSA and CSA were lower in comparison with the control in the first and second cut. Moreover, the NDF fraction was not affected by the biological inoculants in the first and second cut, compared with the control. On the other hand, the NDF fraction in the CSA-group showed significantly lower values in comparison with the control in the first and second cut.

While in the first cut, the concentration of CP of BSA-group was higher in comparison with the CSA-group, no significant differences were observed in the second cut.

Treated silage (biological and chemical) of the first cut showed a higher OMD with 87.0% and 87.9%, respectively, in comparison with the untreated silage (85.3%). In the second cut, no significant differences in OMD between the silage treatments occurred.

In our experiment the year factor significantly affected the chemical composition in both cuts. Concerning the first cut, the contents of CF, ADF, and NDF fractions were lower in 2012 (27.7%, 29.7%, and 50.6%, respectively) than in 2013 (33.6%, 40.2%, and 60.4%, respectively). The contents of DM, CP, and OMD were higher in 2012 (40.9%, 9.5%, and 91.8%, respectively) than in 2013 (31.2%, 8.5%, and 81.6%, respectively). Concerning the results of the second cut, DM content was also higher in 2012 (47.5%) than in 2013 (46.9%). Furthermore, the contents of CP, CF, and ADF were lower in 2012 (8.2%, 29.9%, and 34.3%, respectively) than in 2013 (9.5%, 31.0%, and 36.1%, respectively). The NDF fraction and OMD value did not significantly differ between the years 2012 and 2013.

### Effects of additives on fermentation parameters of grass silage

The application of different silage additives (SA) considerably influenced the quality of silages (Table 5 and 6). The treatment with biological and chemical additives had a favourable effect on pH-values compared with control silages in both cuts. The pH-values of silage with additives were lower (4.0 and 4.2 compared to 4.5 in the first cut, and 4.2 and 4.6 compared to 4.8 in the second cut) after 90 days of ensiling com-



**Table 3.** Chemical composition (%) of cocksfoot silages - First cut 2012-2013.

Factor	DM	CP	CF	ADF	NDF	OMD
<b>Varieties (V)</b>						
Greenly	36.9 ± 0.5 <sup>a</sup>	8.0 ± 0.4 <sup>a</sup>	30.3 ± 1.2 <sup>ab</sup>	34.9 ± 1.8 <sup>abc</sup>	56.3 ± 2.1 <sup>ac</sup>	85.4 ± 2.3 <sup>ab</sup>
Starly	37.4 ± 0.4 <sup>ab</sup>	8.1 ± 0.3 <sup>a</sup>	29.5 ± 1.2 <sup>a</sup>	33.8 ± 2.1 <sup>b</sup>	52.9 ± 2.2 <sup>b</sup>	88.8 ± 2.2 <sup>a</sup>
Sw-Luxor	37.9 ± 1.5 <sup>b</sup>	8.4 ± 0.1 <sup>ab</sup>	31.0 ± 1.1 <sup>ab</sup>	34.6 ± 1.7 <sup>ab</sup>	56.8 ± 2.0 <sup>a</sup>	85.1 ± 2.3 <sup>b</sup>
Otello	38.9 ± 1.9 <sup>c</sup>	9.4 ± 0.2 <sup>c</sup>	30.9 ± 1.0 <sup>ab</sup>	35.5 ± 1.6 <sup>ac</sup>	56.2 ± 1.2 <sup>ac</sup>	87.1 ± 1.3 <sup>ab</sup>
Husar	33.1 ± 1.4 <sup>d</sup>	9.1 ± 0.1 <sup>cd</sup>	30.3 ± 1.0 <sup>ab</sup>	34.8 ± 1.6 <sup>ab</sup>	55.5 ± 1.6 <sup>ac</sup>	87.1 ± 1.9 <sup>ab</sup>
Amera	29.2 ± 0.2 <sup>e</sup>	10.5 ± 0.2 <sup>e</sup>	31.9 ± 0.7 <sup>b</sup>	36.4 ± 1.2 <sup>c</sup>	57.0 ± 1.1 <sup>a</sup>	88.7 ± 2.1 <sup>a</sup>
Dika	36.8 ± 1.4 <sup>a</sup>	9.3 ± 0.1 <sup>cd</sup>	30.3 ± 0.7 <sup>ab</sup>	34.5 ± 1.4 <sup>ab</sup>	53.2 ± 1.1 <sup>b</sup>	88.6 ± 1.4 <sup>a</sup>
Bepro	39.3 ± 2.0 <sup>c</sup>	8.8 ± 0.3 <sup>bd</sup>	30.5 ± 1.1 <sup>ab</sup>	34.4 ± 1.8 <sup>ab</sup>	56.1 ± 1.7 <sup>ac</sup>	85.9 ± 1.5 <sup>ab</sup>
Dana	35.7 ± 1.4 <sup>f</sup>	9.3 ± 0.1 <sup>cd</sup>	29.8 ± 0.6 <sup>a</sup>	34.6 ± 1.2 <sup>ab</sup>	54.6 ± 0.9 <sup>bc</sup>	85.7 ± 0.8 <sup>ab</sup>
Vega	35.7 ± 1.5 <sup>f</sup>	8.8 ± 0.2 <sup>bd</sup>	31.8 ± 1.0 <sup>b</sup>	35.9 ± 1.7 <sup>ac</sup>	56.4 ± 1.7 <sup>ac</sup>	84.9 ± 1.4 <sup>b</sup>
<i>p</i> value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<b>Silage additives (SA)</b>						
US	35.3 ± 0.8 <sup>a</sup>	9.0 ± 0.2 <sup>ab</sup>	31.5 ± 0.6 <sup>a</sup>	35.7 ± 1.0 <sup>a</sup>	56.3 ± 1.0 <sup>a</sup>	85.3 ± 1.1 <sup>a</sup>
BSA	36.1 ± 0.8 <sup>b</sup>	9.1 ± 0.2 <sup>a</sup>	30.4 ± 0.5 <sup>b</sup>	34.7 ± 0.8 <sup>b</sup>	55.9 ± 0.8 <sup>a</sup>	87.0 ± 0.9 <sup>b</sup>
CSA	36.8 ± 0.7 <sup>c</sup>	8.9 ± 0.2 <sup>b</sup>	30.1 ± 0.5 <sup>b</sup>	34.4 ± 0.8 <sup>b</sup>	54.3 ± 0.8 <sup>b</sup>	87.9 ± 0.9 <sup>b</sup>
<i>p</i> value	<0.0001	0.0033	<0.0001	<0.0001	<0.0001	<0.0001
<b>Years (Y)</b>						
2012	40.9 ± 0.5 <sup>a</sup>	9.5 ± 0.1 <sup>a</sup>	27.7 ± 0.2 <sup>a</sup>	29.7 ± 0.2 <sup>a</sup>	50.6 ± 0.3 <sup>a</sup>	91.8 ± 0.5 <sup>a</sup>
2013	31.2 ± 0.4 <sup>b</sup>	8.5 ± 0.1 <sup>b</sup>	33.6 ± 0.2 <sup>b</sup>	40.2 ± 0.2 <sup>b</sup>	60.4 ± 0.3 <sup>b</sup>	81.6 ± 0.4 <sup>b</sup>
<i>p</i> value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
V × SA	<0.0001	0.0016	0.1807	0.0116	0.0468	0.1037
V × Y	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SA × Y	<0.0001	0.3999	0.0009	<0.0001	<0.0001	0.0003
V × SA × Y	<0.0001	0.0011	0.7892	0.7527	0.3955	0.2402

DM: dry matter; CP: crude protein; CF: crude fibre; ADF: acidic detergent fibre; NDF: neutral detergent fibre; OMD: organic matter digestibility. US: untreated silage; BSA: biological silage additives; CSA: chemical silage additives. <sup>a,b,c,d,e</sup> indicate significant differences between values within rows ( $p < 0.05$ ).

pared with untreated silage. Overall, the pH-values in the first cut were lower.

In the first cut, SA treatments improved the LA concentration, where 12.8% was found in the BSA-group, and 9.2% in the CSA-group compared to a 18.6% in the untreated silage. In the second cut, they were 10.0%, 3.9%, and 8.5%, respectively. Nevertheless, the SA treatments concerning LA concentration showed improvement compared with the untreated silage, with the exception of the BSA treatment in the second cut.

The treated silages of the first cut showed lower concentrations of AA in the BSA-group and the CSA-group compared with the untreated silage. On the other hand, the silages treated with biological and chemical additives in the second cut showed higher concentration of AA in comparison with the untreated silage.

In the untreated silage from the first cut, a high concentration of BA was detected (0.1%) in comparison with the treated silages (biological and chemical). Interestingly, concerning varieties, 'Amera' proved to be

a peculiar variety regarding its higher concentration of BA (0.2%) in the first cut.

The use of additives significantly influenced in the concentration of AWE in both cuts. The BSA-group had the highest concentration of AWE in comparison with the CSA-group, as well as the untreated silages (1,636.7 mg KOH/100 g, 1,439.7 mg KOH/100 g, and 1,366.0 mg KOH/100 g, respectively for the first cut; and 1,590.9 mg KOH/100 g, 1,272.1 mg KOH/100 g, and 1,194.3 mg KOH/100 g, respectively for the second cut).

Concerning the year factor, there were significant differences, with lower pH-values for 2013 in both cuts compared with 2012; this was more visible in the second cut. Therefore, there was a substantially lower concentration of LA in the second cut compared to the first cut. Furthermore, the year factor effect was more evident for the first cut, where in 2012 the LA concentration was 9.6%; it was 17.5% in 2013. In general, the year 2012 accounted for lower concentrations of LA in both

**Table 4.** Chemical composition (%) of cocksfoot silages - Second cut 2012-2013.

Factor	DM	CP	CF	ADF	NDF	OMD
<b>Varieties (V)</b>						
Greenly	44.2 ± 0.3 <sup>a</sup>	8.8 ± 0.2 <sup>abd</sup>	30.2 ± 0.4 <sup>a</sup>	35.3 ± 0.3 <sup>abc</sup>	56.2 ± 0.3 <sup>ac</sup>	83.7 ± 0.7
Starly	42.7 ± 0.3 <sup>b</sup>	8.7 ± 0.2 <sup>abc</sup>	30.0 ± 0.4 <sup>a</sup>	35.8 ± 0.5 <sup>ab</sup>	55.0 ± 0.4 <sup>ab</sup>	83.8 ± 0.6
Sw-Luxor	47.1 ± 0.4 <sup>c</sup>	8.6 ± 0.2 <sup>ac</sup>	30.3 ± 0.2 <sup>ab</sup>	36.7 ± 0.4 <sup>b</sup>	56.3 ± 0.2 <sup>ac</sup>	82.5 ± 0.9
Otello	47.3 ± 0.2 <sup>c</sup>	8.9 ± 0.3 <sup>abd</sup>	31.2 ± 0.3 <sup>bc</sup>	36.2 ± 0.5 <sup>ab</sup>	56.6 ± 0.3 <sup>c</sup>	84.5 ± 0.7
Husar	47.3 ± 0.1 <sup>c</sup>	8.4 ± 0.2 <sup>c</sup>	30.3 ± 0.5 <sup>ab</sup>	34.9 ± 0.5 <sup>abc</sup>	54.5 ± 0.3 <sup>b</sup>	85.4 ± 0.4
Amera	49.6 ± 0.3 <sup>d</sup>	9.4 ± 0.2 <sup>c</sup>	29.6 ± 0.3 <sup>a</sup>	34.8 ± 0.5 <sup>abc</sup>	54.2 ± 0.4 <sup>b</sup>	85.6 ± 0.8
Dika	48.0 ± 0.4 <sup>c</sup>	9.0 ± 0.2 <sup>abd</sup>	29.9 ± 0.1 <sup>a</sup>	35.0 ± 0.5 <sup>abc</sup>	54.5 ± 0.2 <sup>b</sup>	85.2 ± 1.1
Bepro	48.3 ± 0.7 <sup>c</sup>	9.1 ± 0.2 <sup>dc</sup>	29.6 ± 0.2 <sup>a</sup>	33.8 ± 0.5 <sup>c</sup>	55.1 ± 0.3 <sup>ab</sup>	84.0 ± 0.7
Dana	49.5 ± 0.2 <sup>d</sup>	8.8 ± 0.3 <sup>abcd</sup>	31.3 ± 0.2 <sup>c</sup>	34.3 ± 0.3 <sup>ac</sup>	56.5 ± 0.3 <sup>c</sup>	83.8 ± 0.5
Vega	47.7 ± 0.1 <sup>ce</sup>	9.1 ± 0.3 <sup>bde</sup>	31.7 ± 0.1 <sup>c</sup>	35.1 ± 0.4 <sup>abc</sup>	57.1 ± 0.2 <sup>c</sup>	83.8 ± 0.7
<i>p</i> value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1346
<b>Silage additives (SA)</b>						
US	47.0 ± 0.3 <sup>a</sup>	8.9 ± 0.1	30.9 ± 0.2 <sup>a</sup>	35.8 ± 0.3 <sup>a</sup>	55.8 ± 0.2 <sup>a</sup>	84.0 ± 0.4
BSA	47.2 ± 0.3 <sup>b</sup>	8.9 ± 0.1	30.2 ± 0.2 <sup>b</sup>	34.9 ± 0.3 <sup>b</sup>	55.8 ± 0.2 <sup>a</sup>	83.9 ± 0.5
CSA	47.4 ± 0.3 <sup>b</sup>	8.8 ± 0.1	30.1 ± 0.2 <sup>b</sup>	34.9 ± 0.3 <sup>b</sup>	55.3 ± 0.2 <sup>b</sup>	84.8 ± 0.3
<i>p</i> value	<0.0001	0.1331	<0.0001	0.0003	0.0139	0.2881
<b>Years (Y)</b>						
2012	47.5 ± 0.3 <sup>a</sup>	8.2 ± 0.1 <sup>a</sup>	29.9 ± 0.1 <sup>a</sup>	34.3 ± 0.2 <sup>a</sup>	55.6 ± 0.2	84.0 ± 0.3
2013	46.9 ± 0.2 <sup>b</sup>	9.5 ± 0.0 <sup>b</sup>	31.0 ± 0.1 <sup>b</sup>	36.1 ± 0.2 <sup>b</sup>	55.6 ± 0.2	84.4 ± 0.4
<i>p</i> value	<0.0001	<0.0001	<0.0001	<0.0001	0.7905	0.3883
V × SA	<0.0001	0.0062	0.0006	0.2122	0.9115	0.8514
V × Y	<0.0001	<0.0001	<0.0001	0.0072	0.1178	0.0844
SA × Y	0.0042	0.7900	0.2886	0.4445	0.0153	0.8259
V × SA × Y	<0.0001	0.0001	0.0001	0.4561	0.0143	0.7689

See legend of Table 3.

cuts. Nevertheless, in 2012, in both cuts, a significantly lower AA concentration occurred, compared to 2013. In the first cut, the concentration of BA 0.1% was significantly higher in 2012 than in 2013; however, in the second cut, this acid was not observed at all. Concerning the first cut, the concentration of AWE in 2012 (1,615.3 mg KOH/100 g) was higher in comparison with 2013 (1,346.2 mg KOH/100 g); but in the second cut, this relationship was inverted (1,146.9 mg KOH/100 g for 2012, and 1,558.0 mg KOH/100 g for 2013).

## Discussion

### Chemical composition of green forage

The chemical composition of green forage is an important starting point for analyses as forage quality is, in part, altered by *in situ* growth conditions and different genotypes seem to respond differently to changing global climate (DaMatta *et al.*, 2010). The results of chemical composition of analysed fresh cocksfoot forages proved that there was a significant

influence of the factor year on the content of DM, CP, CF, ADF, NDF, and OMD in the first cut; and also on the content of CP, CF, and OMD in the second cut. Our results are in concordance with the chemical composition results in different fresh grasses cultivars obtained by Skládanka *et al.* (2012).

In our results, more notably in the first cut, it was shown that the year factor had an important effect regarding the chemical composition. Thus, with lately increasing climatic changes, it is natural that risk management has become the central axis of many climate change assessments, especially due to projected increases in extreme weather events (Kalaugher *et al.*, 2013). Importantly, risk management should weigh not only current, but anticipated changes, for the evaluation of frequency of potential major losses (Yakushev, 2009).

### Effects of additives on chemical composition of grass silage

The DM losses in the first cut compared with the second cut were probably caused by higher fermenta-

**Table 5.** Fermentation characteristics of cocksfoot silages - First cut 2012-2013.

Factor	pH	LA %	AA %	BA %	AWE (KOH) mg/100 g
<b>Varieties (V)</b>					
Greenly	4.2 ± 0.0 <sup>abd</sup>	7.6 ± 0.7 <sup>a</sup>	1.2 ± 0.1 <sup>a</sup>	ND	1,239.4 ± 57.2 <sup>a</sup>
Starly	4.3 ± 0.0 <sup>abd</sup>	8.4 ± 0.8 <sup>a</sup>	1.3 ± 0.1 <sup>ab</sup>	ND	1,294.1 ± 57.0 <sup>a</sup>
Sw-Luxor	4.1 ± 0.1 <sup>c</sup>	13.1 ± 1.6 <sup>b</sup>	1.2 ± 0.1 <sup>a</sup>	ND	1,623.6 ± 96.4 <sup>bd</sup>
Otello	4.2 ± 0.0 <sup>ac</sup>	14.2 ± 1.9 <sup>bc</sup>	0.9 ± 0.1 <sup>c</sup>	ND	1,545.6 ± 59.6 <sup>bc</sup>
Husar	4.2 ± 0.0 <sup>abcd</sup>	14.8 ± 1.3 <sup>cd</sup>	1.3 ± 0.1 <sup>ab</sup>	ND	1,456.4 ± 70.8 <sup>c</sup>
Amera	4.3 ± 0.1 <sup>bd</sup>	14.4 ± 1.3 <sup>bc</sup>	1.2 ± 0.0 <sup>a</sup>	0.2 ± 0.1	1,253.0 ± 48.3 <sup>a</sup>
Dika	4.2 ± 0.0 <sup>ac</sup>	17.2 ± 1.8 <sup>c</sup>	1.2 ± 0.1 <sup>ab</sup>	ND	1,687.3 ± 45.2 <sup>de</sup>
Bepro	4.1 ± 0.1 <sup>c</sup>	13.1 ± 1.2 <sup>b</sup>	1.5 ± 0.1 <sup>de</sup>	ND	1,739.9 ± 112.2 <sup>e</sup>
Dana	4.3 ± 0.1 <sup>d</sup>	16.0 ± 1.8 <sup>de</sup>	1.4 ± 0.1 <sup>bd</sup>	ND	1,448.2 ± 73.7 <sup>c</sup>
Vega	4.2 ± 0.1 <sup>abc</sup>	16.5 ± 1.6 <sup>c</sup>	1.6 ± 0.1 <sup>e</sup>	ND	1,520.2 ± 77.0 <sup>bc</sup>
<i>p</i> value	<0.0001	<0.0001	<0.0001		<0.0001
<b>Silage additives (SA)</b>					
US	4.5 ± 0.0 <sup>a</sup>	18.6 ± 1.1 <sup>a</sup>	1.5 ± 0.1 <sup>a</sup>	0.1 ± 0.0	1,366.0 ± 50.5 <sup>a</sup>
BSA	4.0 ± 0.0 <sup>b</sup>	12.8 ± 0.3 <sup>b</sup>	1.2 ± 0.1 <sup>b</sup>	ND	1,636.7 ± 14.7 <sup>b</sup>
CSA	4.2 ± 0.0 <sup>c</sup>	9.2 ± 0.5 <sup>c</sup>	1.1 ± 0.1 <sup>b</sup>	ND	1,439.7 ± 49.1 <sup>c</sup>
<i>p</i> value	<0.0001	<0.0001	<0.0001		<0.0001
<b>Years (Y)</b>					
2012	4.2 ± 0.0 <sup>a</sup>	9.6 ± 0.3 <sup>a</sup>	1.1 ± 0.0 <sup>a</sup>	0.1 ± 0.0	1,615.3 ± 37.5 <sup>a</sup>
2013	4.2 ± 0.0 <sup>b</sup>	17.5 ± 0.8 <sup>b</sup>	1.5 ± 0.0 <sup>b</sup>	ND	1,346.2 ± 28.3 <sup>b</sup>
<i>p</i> value	0.0047	<0.0001	<0.0001		<0.0001
V × SA	<0.0001	<0.0001	<0.0001		<0.0001
V × Y	<0.0001	<0.0001	<0.0001		<0.0001
SA × Y	<0.0001	<0.0001	<0.0001		<0.0001
V × SA × Y	<0.0001	<0.0001	<0.0001		<0.0001

LA: lactic acid; AA: acetic acid; BA: butyric acid; AWE: acid water extracted. US: untreated silage; BSA: biological silage additives; CSA: chemical silage additives. Indices (<sup>a,b,c,d,e,f</sup>) indicate significant differences ( $p < 0.05$ ) using Scheffé's test. ND: no detected.

tion activity associated with catabolism of cellulose and hemicellulose in inoculated forage grasses. In accordance with different findings, the DM losses of up to 12% are considered a reliable indicator of inoculant activity in silages forage (Driehuis *et al.*, 2001).

In our work, the CSA-group showed significantly higher contents of DM compared to both the control and the BSA-group in the first cut. Moreover, the CSA-group and the BSA-group exhibited a significantly higher content of DM than the untreated silage in the second cut.

In the first cut, the CP content of BSA-group increased in comparison with the control silage. Nevertheless, in the second cut, no statistical differences were observed. Kleinschmit & Kung (2006a) noted that the inoculation with *Lactobacillus buchneri* and *Pediococcus pentosaceus* did not affect the CP value. Moreover, the optimal mean concentration of CP in grass silage is approximately 16.0% of DM, although it can range from 3.9% to 28.2% of DM (Merry *et al.*, 2000).

Our results showed that ADF content was affected by biological silage additives in comparison with untreated silage in both cuts. We assume that the reduction of ADF in the inoculated silages was due to partial hydrolysis of cellulose. Hence, our findings are consistent with the previous reports on the nutritional qualities of different grasses and their mixtures presented by Skládanka *et al.* (2012), where biological inoculants and chemical preservatives were used in some grass silages such as *Lolium perenne*, *Festulolium pabulare*, *Festulolium braunii*; and the mixtures of these with *Festuca rubra* and *Poa pratensis*. Nevertheless, our results differ from the studies reporting a lack of effect of inoculated treatments on fibre degradability in analyses realized on frost corn silages, reported by Mohammadzadeh *et al.* (2012).

The content of NDF in our study was not affected by biological inoculants during fermentation in comparison with the control in both cuts. These findings are consistent with the study realized by Jalč *et al.* (2009), where the content of NDF in cocksfoot silage

**Table 6.** Fermentation characteristics of cocksfoot silages - Second cut 2012-2013.

Factor	pH	LA %	AA %	BA %	AWE (KOH) mg/100 g
<b>Varieties (V)</b>					
Greenly	4.5 ± 0.1 <sup>ac</sup>	8.1 ± 0.7 <sup>a</sup>	0.9 ± 0.1 <sup>a</sup>	ND	1,301.4 ± 59.2 <sup>a</sup>
Starly	4.6 ± 0.1 <sup>ab</sup>	7.9 ± 0.9 <sup>ab</sup>	1.0 ± 0.0 <sup>bdf</sup>	ND	1,265.0 ± 70.1 <sup>a</sup>
Sw-Luxor	4.3 ± 0.0 <sup>c</sup>	8.4 ± 0.5 <sup>a</sup>	1.1 ± 0.0 <sup>c</sup>	ND	1,483.9 ± 49.7 <sup>b</sup>
Otello	4.5 ± 0.1 <sup>de</sup>	8.2 ± 0.5 <sup>a</sup>	1.1 ± 0.1 <sup>cd</sup>	ND	1,429.8 ± 48.1 <sup>b</sup>
Husar	4.4 ± 0.0 <sup>cd</sup>	7.0 ± 0.4 <sup>cd</sup>	1.1 ± 0.1 <sup>ce</sup>	ND	1,443.3 ± 34.4 <sup>b</sup>
Amera	4.5 ± 0.0 <sup>de</sup>	6.5 ± 0.6 <sup>d</sup>	1.2 ± 0.1 <sup>e</sup>	ND	1,449.5 ± 44.7 <sup>b</sup>
Dika	4.6 ± 0.1 <sup>ab</sup>	7.2 ± 0.8 <sup>bc</sup>	1.0 ± 0.1 <sup>b</sup>	ND	1,317.3 ± 89.6 <sup>a</sup>
Bepro	4.7 ± 0.1 <sup>b</sup>	7.0 ± 0.8 <sup>cd</sup>	1.0 ± 0.1 <sup>bf</sup>	ND	1,274.7 ± 87.0 <sup>a</sup>
Dana	4.6 ± 0.1 <sup>ab</sup>	7.3 ± 0.7 <sup>bc</sup>	1.0 ± 0.1 <sup>bdf</sup>	ND	1,311.9 ± 98.8 <sup>a</sup>
Vega	4.7 ± 0.1 <sup>b</sup>	7.0 ± 0.7 <sup>cd</sup>	1.1 ± 0.1 <sup>cdf</sup>	ND	1,247.8 ± 85.2 <sup>a</sup>
<i>p</i> value	<0.0001	<0.0001	<0.0001		<0.0001
<b>Silage additives (SA)</b>					
US	4.8 ± 0.0 <sup>a</sup>	8.5 ± 0.3 <sup>a</sup>	1.0 ± 0.0 <sup>a</sup>	ND	1,194.3 ± 40.1 <sup>a</sup>
BS	4.2 ± 0.0 <sup>b</sup>	10.0 ± 0.2 <sup>b</sup>	1.0 ± 0.0 <sup>b</sup>	ND	1,590.9 ± 15.5 <sup>b</sup>
CSA	4.6 ± 0.0 <sup>c</sup>	3.9 ± 0.3 <sup>c</sup>	1.1 ± 0.0 <sup>c</sup>	ND	1,272.1 ± 39.8 <sup>c</sup>
<i>p</i> value	<0.0001	<0.0001	<0.0001		<0.0001
<b>Years (Y)</b>					
2012	4.6 ± 0.0 <sup>a</sup>	6.6 ± 0.3 <sup>a</sup>	0.8 ± 0.0 <sup>a</sup>	ND	1,146.9 ± 30.0 <sup>a</sup>
2013	4.4 ± 0.0 <sup>b</sup>	8.3 ± 0.2 <sup>b</sup>	1.2 ± 0.0 <sup>b</sup>	ND	1,558.0 ± 20.2 <sup>b</sup>
<i>p</i> value	<0.0001	<0.0001	<0.0001		<0.0001
V × SA	<0.0001	<0.0001	<0.0001		<0.0001
V × Y	<0.0001	<0.0001	<0.0001		<0.0001
SA × Y	<0.0001	<0.0001	<0.0001		<0.0001
V × SA × Y	<0.0001	<0.0001	<0.0001		<0.0001

See legend of Table 5.

did not significantly differ from untreated silage when the silage was treated separately with *Enterococcus faecium* and with *Lactobacillus fermentum*; however, the treatment with *Lactobacillus plantarum* presented significant differences in comparison with the control (Jalč *et al.*, 2009).

In terms of years, it is important to mention that in the year 2012 there was less mean annual precipitation and less mean annual temperature compared to 2013. Nevertheless, the weather change condition, characterized by the co-occurrence of co-varying environmental variables, often affects plant chemical composition differently, as when applied separately (Xu & Zhou, 2006). In our experiments, the weather change condition seems to have altered the DM content in 2012 in both cuts. The CP content was affected in the first cut in 2012 compared to 2013; in the second, the results showed an opposite trend. There is evidence on the decreasing CP and increasing NDF contents induced by seasonal drought presented by Murillo *et al.* (2012). However, Jennings (2010) described opposite results when the rain will leach soluble nutrients (primarily

sugars) from hay resulting in DM loss, increased fiber content, and decreased energy in the forage. Similarly, it is expected that DM losses for the cocksfoot will be low (< 2%) if rainfall occurs when the forage moisture content is high; but DM losses will increase substantially if rainfall occurs when the forage is dry. Therefore, silage producers have to weigh the consequences of delaying conservation, and the risks of damage by rainfall events that can occur before the wilting forage is harvested (Coblentz & Muck, 2012).

On the other hand, the CF and ADF fraction were affected in both cuts in 2013, increasing their contents compared to 2012. Hence, our findings are in good agreement with the study presented by Scarbrough *et al.* (2005) which explained that the rain damage increased all fiber components excluding hemicelluloses in cocksfoot and bermudagrass.

In general, based on expected vegetation changes and known environmental effects on forage protein, carbohydrate, and fibre contents, both positive and negative changes in forage quality are possible as a result of atmospheric and climatic changes (Hatfield



*et al.*, 2008). Regarding the NDF fraction, in the first cut, it was notably affected in the year 2013 in comparison to the year 2012; while in the second cut the NDF content did not show significant year differences. These facts can be due to a different rainfall patterns during the year, where for example, due to precipitation on relatively dry forage, the rain damage on forage is high but the fibre content components (ADF and NDF), which are not water soluble, increase (Jennings, 2010). On the other hand, usually, when linked with the dates harvested, with greater precipitation, there is lower NDF (cell wall) content in the grass (Ramirez, 2015).

### Effects of additives on fermentation parameters of grass silage

The exceptional activities of the biological inoculants include: rapid production of LA, improvement of the aerobic stability of silage due to production of acetic acid (Kleinschmit & Kung, 2006b), detoxification of mycotoxins, as well as inhibition of pathogenic bacteria or fungi undesirable in silage (Li & Nishino, 2011), which also prevent probiotic action (Weinberg *et al.*, 2004).

In agreement with the studies reported by Kung & Ranjit (2001), the application of biological inoculants accelerated the post-ensiling decline in pH, increased the LA concentration, and reduced the concentrations of AA and BA. As soon as a low pH is achieved after ensiling, the aerobic microorganisms and plant enzymes are inhibited more rapidly, which results in reduced proteolysis (Zahiroddini *et al.*, 2004). Undoubtedly, the process of fermentation was positively influenced by the use of biological inoculants. Unlike chemical additives, the biological inoculants increased the concentration of LA, and reduced the pH-value to appropriate values for ensiling.

The post-ensiling drop in pH-value in both cuts in comparison with untreated silage, corresponds to the studies presented by Cherney *et al.* (2006) where the pH of silage tended to be under 4.7, which is considered acceptable for grass silages.

Lactic acid production is essential to obtain high quality silage. Being the most efficient fermentation acid, it decreases the silage pH-value more efficiently than other fermentation products (McDonald *et al.*, 2002). Compared to other fermentation acids (*i.e.*, acetic, propionic, and butyric) in silages, LA is stronger (Khaing *et al.*, 2014). Furthermore, it prevents the increase of undesirable bacteria (Vandenbergh, 1993). Thus, its concentration in silages should be at least 65-70% of the total silage acids (Váradyová *et al.*, 2013). Moreover, Doležal *et al.* (2012) reported that

higher concentrations of fermentation acids, LA in particular, represent an important stress factor in the ruminal digestion, leading to the accumulation of this acid which is known as lactic acidosis (Xu & Ding, 2011).

The decrease of concentrations of LA and AA in silages from the first cut observed in our study was in agreement with the study by Váradyová *et al.* (2013), where similar effects were reported from microbial inoculants compared to untreated silage of cocksfoot. Nevertheless, in the second cut, increased concentrations of these acids were observed; hence, our findings are consistent with studies reported by Jalč *et al.* (2009) once more on cocksfoot.

Concerning our results on butyric acid, no BA was found in well-fermented silages (pH of 4.0-4.2) treated with biological and chemical preservatives, while untreated silages contained a detectable concentration of this acid only in the first cut. These findings are consistent with the study of Ohmomo *et al.* (2002), who presented similar data in untreated grass silages with pH of 4.1-4.2. The occurrence of BA usually is not desired in silage (Danner *et al.*, 2003) because it is responsible of metabolic disorder in dairy cows, world renowned as ketosis (Oetzel, 2007). The development of this acid can be prevented by inhibiting the detrimental effect of oxygen penetration into the silage via the addition of *Lactobacillus buchneri* (inhibitor of aerobic deterioration) or by wilting the forage before ensiling (Martínez-Fernández *et al.*, 2010); it can also be controlled with a quick and strong acidification (Kramer, 2002). Usually, very high contents of BA in untreated silages are the result of clostridial metabolism, resulting from contamination with soil or slurry (Danner *et al.*, 2003). Furthermore, the absence of BA implies that the silage had not been subdued to clostridial fermentation (Krizsan *et al.*, 2012). Hence, we assume that all the silage materials had a minimum or no clostridial contamination, and such contamination could likely explain that in the first cut of untreated silage, a higher concentration of BA (0.1%) was detected.

The acid water extract (AWE) is an indispensable indicator of acidity in the ensiling crops. The compliance of the titration acidity of the water extract with the fermentation acid concentration can be considered as an important indicator of the quality of the fermentation process (Doležal *et al.*, 2008). Concerning the titration acidity in our silages, both the total content of acids and the content of LA were in concordance with Doležal *et al.* (2012), who reported concentrations of AWE of 1,000–1,300 mg KOH/100 g as ideal for grass silages.

The overall weather conditions by means of annual precipitation and mean annual temperature may have induced part of the year factor result. Undoubtedly, our results showed principal differences between the two

years. The year's climate conditions were different for each year; in 2012, 47 mm less annual average precipitation, and 0.5°C less mean annual temperature were measured than in 2013. On the other hand, the timing of rainfall and temperature changes and/or extreme events may induce important differences in ecosystem productivity without changes in annual totals, as these factors determine whether the water will be used by plants and transpired, or will just run off or evaporate (Heimann & Reichstein, 2008). In addition, due to global climate change, it is necessary to test different fodders as silages (Glamočlija *et al.*, 2011).

Our results indicate that inoculating with dual-purpose LA bacteria (homo- and hetero-fermentative) contributed to decrease the concentrations of LA and AA minimizing the pH-value in comparison with the untreated silage in the first cut. Furthermore, in the second cut, the biological inoculants affected positively on the quality of fermentation process, increasing the concentrations of LA, AA, and AWE; and decreasing the pH-value to the appropriate parameters of ensiling, compared with untreated silage. The reduction of pH-values under anaerobic process can be explained by the probiotic effect of LA bacteria (in the ensile crops), whereby this bacterium ferments the water-soluble carbohydrates into organic acids, mainly LA, which reduces the pH ranges (Weinberg, 2008; McDonald *et al.*, 2010).

It is important to point out that silage additives are used to improve the fermentation process (Bolsen *et al.*, 1995); nevertheless, Kung *et al.* (2003) indicated that additives do not always enhance fermentation, and this is particularly true when the control silages undergo excellent fermentations.

Additionally, in terms of ensilability, 'Greenly' and 'Starly' varieties from the first cut, and 'Greenly', 'Sw-Luxor', and 'Otello' varieties from the second cut showed very good results; thus, they can be considered preferable for ensiling because their pH-values and LA and AA concentrations had ideal parameters for the fermentation process in cocksfoot silage (10% for LA, and 1.5-3% for AA), according to Doležal *et al.* (2012).

In summary, these results suggest that the treatment of cocksfoot with biological inoculant effectively influenced on the chemical composition and fermentation process after ensiling. Hence, it can be concluded that the combination of homo- and hetero-fermentative LA bacteria in the BSA-group of our study had a positive effect on cocksfoot silage in comparison with the untreated silage. Obtaining the performance of the cocksfoot varieties at a conventional scale will be desirable in order to verify our results at the laboratory scale. More studies aimed to improve the quality of the fermentation process in cocksfoot silage are still necessary.

## Acknowledgments

Our sincere gratitude to the reviewers for their valuable comments and for assessing the manuscript, and to Dr. Peter R. Lemkin, PhD., for linguistic revision.

## References

- AOAC, 1980. Official methods of analysis, 13th ed. Association of Official Analytical Chemists, Washington, DC, USA. 1040 pp.
- Bolsen KK, Ashbell G, Wilkinson JM, 1995. Silage additives. In: Biotechnology in animal feeds and feeding; Wallace RJ, Chesson A (eds.). pp: 33-54. VCH Press, Weinheim. <http://dx.doi.org/10.1002/9783527615353.ch3>
- Charmley E, 2001. Towards improved silage quality – A review. *Can J Anim Sci* 81(2): 157-168. <http://dx.doi.org/10.4141/A00-066>
- Cherney DRJ, Alessi MA, Cherney JH, 2006. Influence of grass species and sample preparation on ensiling characteristics. *Crop Sci* 46(1): 256-263. <http://dx.doi.org/10.2135/cropsci2005.04-0032>
- Coblentz WK, Muck RE, 2012. Effects of natural and simulated rainfall on indicators of ensilability and nutritive value for wilting alfalfa forages sampled before preservation as silage. *J Dairy Sci* 95(11): 6635-6653. <http://dx.doi.org/10.3168/jds.2012-5672>
- ČSN 467092 Standards (1997). Testing method for feeding stuffs. Praha, CZ. [In Czech].
- DaMatta FM, Grandis A, Arenque BC, Buckeridge MS, 2010. Impacts of climate changes on crop physiology and food quality. *Food Res Int* 43 (7): 1814-1823. <http://dx.doi.org/10.1016/j.foodres.2009.11.001>
- Danner H, Holzer M, Mayrhuber E, Braun R, 2003. Acetic acid increases stability of silage under aerobic conditions. *Appl Environ Microbiol* 69(1): 562-567. <http://dx.doi.org/10.1128/AEM.69.1.562-567.2003>
- Doležal P, 2002. Vliv přídavku *Lactobacillus plantarum* DSM 12771 na kvalitu siláží silně zaváděle vojtěšky a trávy. *Acta Univ Agric et Silvicae Mend Brun* 51: 37-44.
- Doležal P, Zeman L, Skládanka J, 2008. Effect of supplementation of chemical preservative on fermentation process of lupine silage. *Slovak J Anim Sci* 41 (1): 30-38.
- Doležal P, Dvořáček J, Loučka R, Mikyska F, Mudřík Z, Opitz von Boberfeld W, Prokeš K, Příkryl J, Skládanka J, Straková E, et al., 2012. Konzervace krmiv a jejich využití ve výživě zvířat. Baštan, Olomouc, CZ. 307 pp.
- Driehuis F, Oude Elferink SJWH, Van Wixselaar PG, 2001. Fermentation characteristics and aerobic stability of grass silage inoculated with *Lactobacillus buchneri* with or without homofermentative LAB. *Grass Forage Sci* 56(4): 330-343. <http://dx.doi.org/10.1046/j.1365-2494.2001.00282.x>
- Filya I, Muck R, Contreras-Govea F, 2007. Inoculant effects on alfalfa silage: fermentation products and nutritive value. *J Dairy Sci* 90(11): 5108-5114. <http://dx.doi.org/10.3168/jds.2006-877>

- Glamočlija Đ, Janković S, Rakić S, Maletić R, Ikanović J, Lakić Ž, 2011. Effects of nitrogen and harvesting time on chemical composition of biomass of sudan grass, fodder sorghum, and their hybrid. *Turk J Agric For* 35(2): 127-138.
- Hatfield J, Boote K, Fay P, Hahn L, Izaurralde C, Kimball BA, Mader T, Morgan J, Ort D, Polley D, Thomson A, Wolfe D, 2008. Agriculture. In: The effects of climate change on agriculture, land resources, water resources, and biodiversity in the United States; Backlund P, Janetos A, Schimel D (eds.). pp: 22-74. U.S. Climate Change Science Program, Washington, DC.
- Heimann M, Reichstein M, 2008. Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature* 451(176): 289-292. <http://dx.doi.org/10.1038/nature06591>
- Huhtanen P, Rinne M, Nousiainen JI, 2007. Evaluation of the factors affecting silage intake of dairy cows: a revision of the relative silage dry matter intake index. *Animal* 1 (5): 758-770. <http://dx.doi.org/10.1017/S175173110773673X>
- Jalč D, Lauková A, Simonová M, Varadyová Z, Homolka P, 2009. The use of bacterial inoculants for grass silage: their effects on nutrient composition and fermentation parameters in grass silages. *Czech J Anim Sci* 54(2): 84-91.
- Jennings JA, 2010. Rainfall effects on wilting forages. *Agriculture and Natural Resources*. Univ. Arkansas, Div. Agric. FSA3090, 8 pp. <https://www.uaex.edu/publications/pdf/FSA-3090.pdf>.
- Kalaugher E, Bornman JF, Clark A, Beukes P, 2013. An integrated biophysical and socio-economic framework for analysis of climate change adaptation strategies: the case of a New Zealand dairy farming system. *Environ Modell Softw* 39: 176-187. <http://dx.doi.org/10.1016/j.envsoft.2012.03.018>
- Khaing KT, Loh TC, Ghizan S, Halim RA, Samsudin AA, 2014. Effect of different particle lengths on the bacterial population, fermentation profiles and nutritive value of whole maize plant silage. *Livest Res Rural Dev* 26: 197. <http://www.lrrd.org/lrrd26/11/khai26197.html>.
- Kleinschmit DH, Kung L Jr, 2006a. The effects of *Lactobacillus buchneri* 40788 and *Pediococcus pentosaceus* R1094 on the fermentation of corn silage. *J Dairy Sci* 89 (10): 3999-4004. [http://dx.doi.org/10.3168/jds.S0022-0302\(06\)72443-2](http://dx.doi.org/10.3168/jds.S0022-0302(06)72443-2)
- Kleinschmit DH, Kung L Jr, 2006b. A meta-analysis of the effects of *Lactobacillus buchneri* on the fermentation and aerobic stability of corn and grass and small-grain silages. *J Dairy Sci* 89 (10): 4005-4013. [http://dx.doi.org/10.3168/jds.S0022-0302\(06\)72444-4](http://dx.doi.org/10.3168/jds.S0022-0302(06)72444-4)
- Kramer W, 2002. Neue entwicklungen und strategien im bereich der silierzusätze. Preconf 8 Alpenländisches Expertenforum, Bundesanstalt für Alpenländische Landwirtschaft, Gumpenstein (Austria), Apr 9-10. pp: 27-30.
- Krizsan SJ, Randby ÁT, Westad F, 2012. Effect of acetic acid, caproic acid and tryptamine on voluntary intake of grass silage by growing cattle. *Grass Forage Sci* 67 (3): 361-368. <http://dx.doi.org/10.1111/j.1365-2494.2012.00852.x>
- Kung L Jr, Ranjit NK, 2001. The effect of *Lactobacillus buchneri* and other additives on the fermentation and aerobic stability of barley silage. *J Dairy Sci* 84(5): 1149-1155. [http://dx.doi.org/10.3168/jds.S0022-0302\(01\)74575-4](http://dx.doi.org/10.3168/jds.S0022-0302(01)74575-4)
- Kung L Jr, Stokes MR, Lin CJ, 2003. Silage additives. In: *Silage science and technology*; Buxton DR, Muck RR, Harrison JH (eds.). pp: 305-360. Am Soc Agron, Madison, WI, USA.
- Li Y, Nishino N, 2011. Bacterial and fungal communities of wilted Italian ryegrass silage inoculated with and without *Lactobacillus rhamnosus* or *Lactobacillus buchneri*. *Lett Appl Microbiol* 52 (4): 314-321. <http://dx.doi.org/10.1111/j.1472-765X.2010.03000.x>
- Lindner R, Lema M, Garcia A, 2004. Extended genetic resources of *Dactylis glomerata* ssp. *izcoi* in Galicia (Northwest Spain). *Genet Resour Crop Evol* 51 (4): 437-442. <http://dx.doi.org/10.1023/B:GRES.0000023457.30193.63>
- Martínez-Fernández A, Soldado A, Vicente F, Martínez A, de la Roza-Delgado B, 2010. Wilting and inoculation of *Lactobacillus buchneri* on intercropped triticale-fava silage: effects on nutritive, fermentative and aerobic stability characteristics. *Agr Food Sci* 19(4): 302-312. <http://dx.doi.org/10.2137/145960610794197597>
- McDonald P, Edwards RA, Greenhalgh JFD, Morgan CA, 2002. *Animal nutrition*. Prentice Hall, NJ, USA. 693 pp.
- McDonald P, Greenhalgh JFD, Morgan CA, Edwards R, Sinclair L, Wilkinson R, 2010. *Animal nutrition*. Pearson Education Limited, Harlow, UK. 714 pp.
- Merry RJ, Jones R, Theodorou MK, 2000. The conservation of grass. In: *Grass: its production and utilization*; Hopkins A (ed.). pp: 196-228. Blackwell Sci. Ltd., Oxford, UK.
- Míka V, Kohoutek A, Odstrčilová V, 2002. Characteristics of important diploid and tetraploid subspecies of *Dactylis* from point of view of the forage crop production. *Rostlinná Výroba* 48 (6): 243-248.
- Mohammadzadeh H, Khorvash M, Ghorbani GR, Yang WZ, 2012. Frosted corn silage with or without bacterial inoculants in dairy cattle ration. *Livest Sci* 145(1-3): 153-159. <http://dx.doi.org/10.1016/j.livsci.2012.01.011>
- Murillo M, Herrera E, Carrete FO, Ruiz O, Serrato JS, 2012. Chemical composition, in vitro gas production, ruminal fermentation and degradation patterns of diets by grazing steers in native range of North Mexico. *Asian-Aust J Anim Sci* 25 (10): 1395-1403. <http://dx.doi.org/10.5713/ajas.2012.12281>
- Oetzel GR, 2007. Herd level ketosis – diagnosis and risk factors. Preconf 7C on Dairy Herd Problem Investigation Strategies: Transition Cow Troubleshooting, Vancouver (Canada), Sept 19. pp: 61-91.
- Ohmomo S, Tanaka O, Kitamoto HK, Cai Y, 2002. Silage and microbial performance, old story but new problems. *Jpn Agr Res Q* 36(2): 59-71. <http://dx.doi.org/10.6090/jarq.36.59>
- Pozdišek J, Loučka R, Macháčová E, 2003. Digestibility and nutrition value of grass silages. *Czech J Anim Sci* 48 (9): 359-364.
- Ramirez-Lozano R, 2015. *Grass nutrition*. Palibrio, Nuevo León, Mexico. 347 pp.
- Sanada Y, Gras M Ch, van Santen E, 2010. Cocksfoot. In: *Fodder crops and amenity grasses*; Boller B, Posselt UK, Veronesi F (eds.). pp: 317-328. Springer, New York. [http://dx.doi.org/10.1007/978-1-4419-0760-8\\_13](http://dx.doi.org/10.1007/978-1-4419-0760-8_13)

- Sanderson MA, Skinner RH, Elwinger GF, 2002. Seedling development and field performance of prairiegrass, grazing brome grass, and orchardgrass. *Crop Sci* 42(1): 224-230. <http://dx.doi.org/10.2135/cropsci2002.0224>
- Sariçiçek ZB, Kiliç Ü, 2009. The effects of different additives on silage gas production, fermentation kinetics and silage quality. *Ozean J Appl Sci* 2(1): 11-18.
- Scarborough DA, Coblenz WK, Humphry JB, Coffey KP, Daniel TC, Sauer TJ, Jennings JA, Turner JE, Kellogg DW, 2005. Evaluation of dry matter loss, nutritive value, and in situ dry matter disappearance for wilting orchardgrass and bermudagrass forages damaged by simulated rainfall. *Agron J* 97(2): 604-614. <http://dx.doi.org/10.2134/agronj2005.0604>
- Skládanka J, Nedělník J, Doležal P, Lindušková H, Nawrath A, 2012. Influence of forage species and preservation additives on quality and mycotoxins safety of silages. *Acta Fytotechnica et Zootechnica*. 15: 50-54.
- Vandenbergh PA, 1993. Lactic acid bacteria, their metabolic products and interference with microbial growth. *FEMS Microbiol Rev* 12 (1-3): 221-238. <http://dx.doi.org/10.1111/j.1574-6976.1993.tb00020.x>
- Váradyová Z, Jalč D, Lauková A, Mihalíková K, Homolka P, 2013. Effects of microbial inoculants *Enterococcus faecium* EF2/3s and EF26/42 on microbial, chemical, and fermentation parameters in grass silage. *Turk J Agric For* 37(3): 344-351.
- Vlková E, Rada V, Bunešová V, Ročková Š, 2012. Growth and survival of lactic acid bacteria in lucerne silage. *Folia Microbiol* 57(4): 359-362. <http://dx.doi.org/10.1007/s12223-012-0142-5>
- Weinberg ZG, 2008. Preservation of forage crops by solid-state lactic acid fermentation-ensiling. In: *Current developments in solid-state fermentation*; Pandey A, Soccol CR, Larroche C (eds.). pp: 443-467. Springer, Berlin. [http://dx.doi.org/10.1007/978-0-387-75213-6\\_19](http://dx.doi.org/10.1007/978-0-387-75213-6_19)
- Weinberg ZG, Muck RE, Weimer PJ, Chen Y, Gamburg M, 2004. Lactic acid bacteria used in inoculants for silage as probiotics for ruminants. *Appl Biochem Biotechnol* 118(1-3): 1-9. <http://dx.doi.org/10.1385/ABAB:118:1-3:001>
- Xu Y, Ding Z, 2011. Physiological, biochemical and histopathological effects of fermentative acidosis in ruminant production: a minimal review. *Span J Agric Res* 9 (2): 414-422. <http://dx.doi.org/10.5424/sjar/20110902-177-10>
- Xu ZZ, Zhou GS, 2006. Combined effects of water stress and high temperature on photosynthesis, nitrogen metabolism and lipid peroxidation of a perennial grass *Leymus chinensis*. *Planta* 224(5): 1080-1090. <http://dx.doi.org/10.1007/s00425-006-0281-5>
- Yakushev P, 2009. Risk analysis as the basis for evaluating the consequences of climate changes in agriculture. *Russ Agric Sci* 35(5): 355-358. <http://dx.doi.org/10.3103/S1068367409050206>
- Zahiroddini H, Baah J, Absalom W, Mcallister TA, 2004. Effect of an inoculant and hydrolytic enzymes on fermentation and nutritive value of whole crop barely silage. *Anim Feed Sci Technol* 117(3-4): 317-330. <http://dx.doi.org/10.1016/j.anifeedsci.2004.08.013>