

Full Length Research Paper

# Effect of the technology of the additional sowing of drought-resistant clover-grass mixture and silage additives on fermentation process quality and nutritive value of baled grass silages

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Additional sowing is able to increase quality of grass stand. Consecutive use of silage additives ensures successful silage process. Additionally sown were semi-natural grass stands in the Czech Republic. Technologies of additional sowing differ in the degree of disruption of the original sward. The mixture that was used consisted of *Arrhenatherum elatius*, *Festulolium pabulare*, *Dactylis glomerata* and *Lotus corniculatus*. Wilted herbage (5 to 24 h wilting) was chopped to a length of 25 mm and ensiled into bales of 1230 mm in diameter. Pressed bales were twice wrapped in a foil of 750 mm in width. They were treated with the probiotic and probio-enzymatic additive. After 90 days silages from the stands with additional sowing exhibited lower ( $P < 0.01$ ) contents of acetic ( $7.0 \text{ g kg}^{-1} \text{ DM}$ ) and butyric ( $7.1 \text{ g kg}^{-1}$ ) acids in comparison with control stand ( $12.1$ , respectively  $8.2 \text{ g kg}^{-1} \text{ DM}$ ) The additional sowing also resulted in the increased ( $P < 0.01$ ) content of acid ADF ( $375.7 \text{ g kg}^{-1} \text{ DM}$ ) and in the increased ( $P < 0.05$ ) content of NEL ( $5.58 \text{ g kg}^{-1} \text{ DM}$ ) in comparison with control stand ( $351.6$ , respectively  $5.52 \text{ g kg}^{-1} \text{ DM}$ ). The use of the probiotic additive decreased ( $P < 0.01$ ) pH ( $4.50$ ) and increased ( $P < 0.01$ ) AWE ( $1025.9 \text{ mg } 100 \text{ g}^{-1}$ ) as well as the content of lactic acid ( $57.6 \text{ g kg}^{-1} \text{ DM}$ ) in comparison with control silage ( $4.98$ ,  $798 \text{ mg } 100 \text{ g}^{-1}$ ,  $34.2 \text{ g kg}^{-1} \text{ DM}$ ). The treated silages contained less ( $P < 0.05$ ) butyric acid.

**Key words:** Silages of wilted forage, probiotic additive, probio-enzymatic additive, quality of silage fermentation process, organic nutrients, semi-natural grass stand, grass stand regeneration.

## INTRODUCTION

Winter feed ration is based on the conserved fodder from the first cut and serves also as additional feed in autumn. Compared with haymaking, ensiling represents a much lower weather risk, lower labour costs and less conservation losses (Opitz, 2005). Different ensiling techniques contribute to varying fermentation quality (Krizsan and Randby, 2007). Microbial contamination may affect mammary gland apoptis (Sláma et al., 2009).

The variation in the fermentation quality of grass silages affects the voluntary feed intake in cattle (Huhtanen et al., 2002). Thanks to the suitable selection of lactic acid bacteria, biological inoculants have a beneficial effect on the improvement of the fermentation quality of silages and decrease the total loss nutrients (Honig and Pahlow, 1986; Wrobel and Zastawny, 2004; Jatkauskas et al., 2010). Lactic acid bacteria containing inoculants are often used as silage additives to enhance the lactic acid fermentation, hence, to better preserve the ensiled material. Bacterial inoculants have advantages over chemical additives because they are easy to use, safe, they do not pollute the environment and are regarded as

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natural product (Muck et al., 2007; Tyrolová and Výborná, 2008; Jalč et al., 2009). Jones and Fychan (1995) state that great differences of fermentation quality in pressed silages, especially from fresh biomass, are primarily due to inappropriate dry matter content. Important advantage of ensiling technology using the method of wrapped bales is namely the speed of wrapping the biomass and creating the anaerobic environment, and in such way reducing also the effect of anthropogenic factor (Wilhelm and Wurm, 1999; Keller and Nonn, 1995; Nonn and Keller, 1993). Each bale represents in fact an independent silo, closed in elastic foils, creating inside the bale conditions favourable for the successful conserving process. For pressed silages of good final quality, decisive is also the optimum dry matter content that should range from 40 to 50% (Nonn and Keller, 1993; Keller and Nonn, 1995; Jonsson et al., 1990 and others). In silages with the dry matter content higher than 50%, warming and high risk occur for silage getting mouldy (Jones and Fychan 1995, Keller and Neitz, 1993 and others). Jonsson et al. (1990) recommend the dry matter content higher than 35% also to prevent the clostridial activity in silages. Kennedy (1989) and Weissbach (1993) state that a disadvantage of this technology is primarily the lower measure weight of silages and the relatively large measure surface. These facts can be important factors of certain risk for anaerobic instability and getting mouldy of silages incorrectly wrapped or in disturbed foils. Weissbach (1993) recommends reassessing this technology critically with respect to the living environment.

Grassland management and weather conditions influence ensiling (Krizsan and Randby, 2007). Individual graminaceous species show great differences in their ensiling capacity (Holúbek et al., 2007). Drought resistance becomes increasingly topical at the present in connection with climate changes. Interspecific hybrids of *Festulolium* spp. combine the persistence of *Festuca* spp. genus with the high forage quality of *Lolium* spp. genus (Casler et al., 2002). Holúbek et al. (2007) found out that the quality of *Dactylis glomerata* sharply decreased if the harvest date of the first cut was overshot. *Arrhenatherum elatius* (L.) P. Beauv is a representative of drought-resistant species (Dostálek and Frantík, 2008). Drought-tolerant are *Lotus* species, too. Characteristic is their ability to establish symbiotic interactions with nitrogen-fixing bacteria (Gibson, 2009). Condensed tannins have been shown to limit proteolysis in *Lotus corniculatus* during ensiling, positive correlation between the concentration of condensed tannins and reduce proteolysis (Marley et al., 2006). Grass stands can be regenerated both by ploughing the old sward and by additional sowing into the partly disturbed sward. Technologies of additional sowing differ according to the rate of the original grass sward disturbance. Treated by rotavator may be narrow gaps, broader furrows or the entire original grass sward. Another possibility is a mere

spreading of seeds with the subsequent use of land leveller.

The goal of this paper is to evaluate silage quality after the additionally sown with a mixture of drought resistant species *F. pabulare*, *A. elatius*, *D. glomerata* and *L. corniculatus*. The experimental hypothesis counted on the effect of additional sowing to increase the content of organic nutrients in silages, possibly on the effect of additional sowing to affect the content of fermenting acids. The use of inoculants should contribute to the increased lactic acid content, to minimize the content of butyric acid (which should practically not occur in silages) and to decrease pH.

## MATERIALS AND METHODS

The field experiment was conducted in Helvíkovice and Bartošovice (north-eastern Bohemia) and established on 5 May 2008 at an altitude of 440 m a.s.l., respectively 650 m a.s.l. in three replicates. Soil contents of minerals on the site in Helvíkovice were 44 mg.kg<sup>-1</sup> P, 133 mg.kg<sup>-1</sup> K, 195 mg.kg<sup>-1</sup> Mg and 6672 mg.kg<sup>-1</sup> Ca and the pH value was 6.5. Soil class was moderate soil. Soil contents of minerals on the site in Bartošovice were 133 mg.kg<sup>-1</sup> P, 472 mg.kg<sup>-1</sup> K, 1830 mg.kg<sup>-1</sup> Ca and the pH value was 5.6. Soil class was moderate soil According to Walter (1957), weather conditions of the three experimental years (2004 to 2007) are in Figures 1 and 2. The size of experimental plots was 0.9 ha (20 × 450 m). There were 5 sowing treatments (for each treatment was done random repeat measurement): (1) control (no seeding), (2) additionally sown without grass stand disruption (Lehner), (3) additionally sown with strip seed drill (SSD), (4) additionally sown with Horsch exaktor SE3 (Exaktor), and (5) additionally sown with Horsch disk pronto 3DC (Disk). Lehner (Lehner Super Vario, Lehner GmbH, Germany) represents the technology of seed spreading onto the original grass sward. Working width is 2 to 9 m and the hopper box volume is 70 L. In our experiment, the seed drill was employed in combination with a weeder whose working width was 6 m. SSD (SE 2 – 024, SOR Libchavy, Czech Republic) is a technology producing grooves of 4 cm in width; its working width is 1.65 m, number of rotary discs 12, row spacing 15 cm, sowing depth max. 4 cm, hopper box volume 200 L, brush seeding apparatus. Exaktor (sowing exaktor Horsch SE 3, HORSCH Maschinen GmbH, Germany) ploughs the entire original grass sward; its working width is 3.1 m, the soil in front of the seeding apparatus is treated by a rotary reel with flat working elements, sowing depth is adjustable 0 to 15 cm and the hopper box volume is 500 L. The function is based on a shallow removal of the upper soil layer with a simultaneous whole-area sowing during which the seeds are laid under a stream of earth into the same depth onto a solid and flat seed bed. Disc (Horsch Pronto 3 DC Horsch Maschinen GmbH, Germany) disrupts the original grass sward only partly. The machine working width is 3 m, number of drill coulters 20, row spacing 15 cm, hopper volume 2800 L and the seeding apparatus is driven by a hydraulic blower. Two rows of discs disrupt the soil surface to adjusted depth, pneumatic bellows level and compact the earth and TurboDisc furrow opener with an integrated tracing wheel supports the precise placement of the seed onto the seed bed, which is followed by even harrowing and covering of all seed rows. Additionally sown was carried out with a mixture of *F. pabulare* (cv. Felina, 14.5 kg ha<sup>-1</sup>), *D. glomerata* (cv. Vega, 5 kg ha<sup>-1</sup>), *A. elatius* (cv. Median, 14.5 kg ha<sup>-1</sup>) and *L. corniculatus* (cv. Taborsky, 1.5 kg ha<sup>-1</sup>). The shares of species additionally sown in the evaluated years 2009 and 2010 are presented in Tab. 1. Biomass from the first cut was harvested in

**Table 1.** Projective dominance (%) of additional sowing species and projective dominance of grasses, clovers and other herbs on the experiment stations Helvíkovice and Bartošovice in the first and second year after additional sowing.

Species	2009					2010				
	N	L	S	E	D	N	L	S	E	D
Helvíkovice										
<i>Festulolium pabulare</i>		1	3	3	1		5	1	2	4
<i>Arrhenatherum elatius</i>				6	2		1	10	9	8
<i>Dactylis glomerata</i>	4	8	7	7	10	7	6	6	7	6
Grasses	55	48	45	53	45	33	32	41	40	45
<i>Lotus corniculatus</i>				1	+		2	6		5
Clovers	15	10	17	11	15	25	30	20	19	10
Other herbs	26	37	38	31	32	40	35	33	36	39
Bartošovice										
<i>Festulolium pabulare</i>		+	2	1	4		1		1	3
<i>Arrhenatherum elatius</i>		+	3	12	2		3	5	10	3
<i>Dactylis glomerata</i>	8	8	9	7	9	10	7	8	6	5
Grasses	40	35	33	40	40	50	35	32	28	24
<i>Lotus corniculatus</i>				2						
Clovers	20	35	38	22	30	10	28	30	40	50
Other herbs	36	27	27	33	28	37	35	37	31	25

N – No seeding, L – Lehner, S – SSD, E – Exaktor, D – Disk; + projective dominance of species < 1%.

mid-May (Helvíkovice), respectively at the beginning of June (Bartošovice) and used for ensiling. Dominant grass species were in the phase of early earing. The stand was cut by reel field chopper (mower Lely Splendimo Model 280 MC, stubble height 5 cm). On the site in Helvíkovice, the stand was wilted for 24 h and twice turned in the course of wilting (Lely Lotus Stabulo and side-delivery rake Deutz Fafr Model SU 71-31). On the site in Bartošovice, the stand wilting time was only 5 h and during that time, the stand was once turned. Wilted forages were chopped by a conventional forage chopper to a length of 25 mm and ensiled without any additive, with a bacterial inoculant, and with a probio-enzymatic additive (for each treatment was done random repeat measurement). Commercial bacterial inoculants supplied by Medipharm were used as additives: preservative bacterial inoculant (Microsil, 0.1 g t<sup>-1</sup>, MEDIPHARM CZ, s.r.o., Czech Republic) and probio-enzymatic inoculant (Goldzym, 0.15 g t<sup>-1</sup>, MEDIPHARM CZ, s.r.o., Czech Republic). Active substance of the first bacterial additive were lactic acid bacteria (LAB) - *Enterococcus faecium* (CCM 6226), *Lactobacillus lactis*, *Pediococcus pentosaceus* (CM 3770), *Lactobacillus planatarum* (CCM 3769), *Lactobacillus casei* (CCM 3775) at a total concentration of bacteria 7.5x10<sup>10</sup> CFU.g<sup>-1</sup>. Active substance of the bacterial enzymatic additive were the same LAB and enzymes composed of cellulase and hemicellulase with the activity 28 000 nkat. The additives were dissolved in water. The control silage was treated with an equivalent amount of water without additives. The silages were baled (bale diameter 1230 mm) with using round-baler Welger RP420 with a variable bale chamber. Pressed bales were twice wrapped with mesh fabric wide 1230 mm and subsequently twice wrapped on the AUTOWRAP wrapping machine (Tanco Autowrap, Ireland) with using the ASPLA foil (Spain) 750 mm in width. Silages sampled 90 days after the beginning of conservation were assessed for organic acids and content of nutrients. The content of nutrients was established according to the norm of the Czech Standard Institute (Anonymous, 1997). Silage DM was determined by oven drying at 60°C to a

constant weight. The content of crude protein was determined according to the Kjeldhal method, (N x 6.25) using a Kjeltec 2300 Analyser unit (FOSS Kjeltec™ 2300). Ankom<sup>220</sup> Fiber analyzer was used to analyze the fibre content according to AOAC (2005). Silage pH was measured in 100 g of fresh silage diluted in 1000 ml of 2 ml toluene added by using InoLab WTW pH 197 pH meter. Lactic acid (LA), acetic acid (AA) and butyric acid (BA) were analyzed according to Kvasnička (2000) on the Ionosep 2003 analyzer. The statistic evaluation was carried out by applying the multivariate analysis and a subsequent verification by the Fischer test.

## RESULTS

Average DM content in silages was higher (P<0.01) on the site in Helvíkovice (485.1 g kg<sup>-1</sup>) than on the site in Bartošovice (233.2 g.kg<sup>-1</sup>) (Table 2). The markedly different DM contents on the assessed sites affected the fermentation process quality. Silages from the site in Bartošovice showed a higher (P < 0.01) content of fermenting acids due to vigorous fermentation process and hence a lower (P < 0.01) pH value. The different concentration of fermenting acids was documented also by the value of titratable acidity of water extract (AWE), which corresponded to the titratable value of fermenting acids. The higher degree of grass stand wilting on the site in Helvíkovice caused a pronounced reduction of not only the production of volatile fatty acids(VFA) but even a reduced production of lactic acid itself. In spite of these facts, the silage exhibited a more favourable LA/AA ratio (7.04) than the silage in Bartošovice (5.87) (Table 2). The

**Table 2.** Influence of site, technology of the additional sowing and silage additives on the dry matter (DM) content and quality of silage fermentation process (pH, acidity water extract - AWE, lactic acid - LA, acetic acid - AA, butyric acid - BA, LA/AA) in the years 2009 and 2010.

	DM g kg <sup>-1</sup> DM	pH	AWE mg 100g <sup>-1</sup>	LA g kg <sup>-1</sup> DM	AA g kg <sup>-1</sup> DM	BA g kg <sup>-1</sup> DM	LA/AA
<b>Site</b>							
Helvíkovice	485.1 <sup>a</sup>	4.83 <sup>a</sup>	889.1 <sup>a</sup>	33.5 <sup>a</sup>	4.3 <sup>a</sup>	0.6 <sup>a</sup>	7.04
Bartošovice	233.2 <sup>b</sup>	4.48 <sup>b</sup>	1002.5 <sup>b</sup>	67.7 <sup>b</sup>	13.6 <sup>b</sup>	12.6 <sup>b</sup>	5.87
Level of significance	0.00000	0,00131	0,03330	0,00000	0,00000	0.00000	0,19244
<b>Additionally sown</b>							
Control	337.5	4.72	888.3	50.1	12.1 <sup>a</sup>	8.2 <sup>a</sup>	4.13 <sup>a</sup>
Lehner	356.4	4.65	919.1	53.0	9.2 <sup>ab</sup>	7.1 <sup>a</sup>	6.09 <sup>ab</sup>
SSD	353.1	4.67	945.2	46.9	9.6 <sup>ab</sup>	7.1 <sup>a</sup>	6.32 <sup>ab</sup>
Exaktor	360.8	4.70	952.5	47.7	7.0 <sup>b</sup>	7.4 <sup>a</sup>	7.36 <sup>b</sup>
Disk	352.6	4.51	1028.3	55.0	7.9 <sup>b</sup>	3.0 <sup>b</sup>	8.18 <sup>b</sup>
Level of significance	0.90763	0.24369	0.08956	0.59478	0.01439	0.01555	0.00080
<b>Additive</b>							
Control	363.1	4.98 <sup>a</sup>	798.0 <sup>a</sup>	34.2 <sup>a</sup>	10.1	10.2 <sup>a</sup>	4.50 <sup>a</sup>
Bacterial	358.3	4.50 <sup>b</sup>	1025.9 <sup>b</sup>	57.6 <sup>b</sup>	8.2	5.7 <sup>b</sup>	8.00 <sup>b</sup>
Bacterial-enzymatic	336.4	4.52 <sup>b</sup>	994.1 <sup>ab</sup>	57.4 <sup>b</sup>	9.1	4.5 <sup>b</sup>	6.62 <sup>ab</sup>
Level of significance	0.57201	0.00140	0.00318	0.00133	0.06860	0.04756	0.00040
<b>Year</b>							
Level of significance	0.00003	0.00000	0.00017	0.02186	0.13602	0.69578	0.00095
R	0.8929	0.7086	0.6357	0.7209	0.8283	0.7229	0.6869
R <sup>2</sup>	0.7973	0.5022	0.4041	0.4812	0.6861	0.5226	0.4718

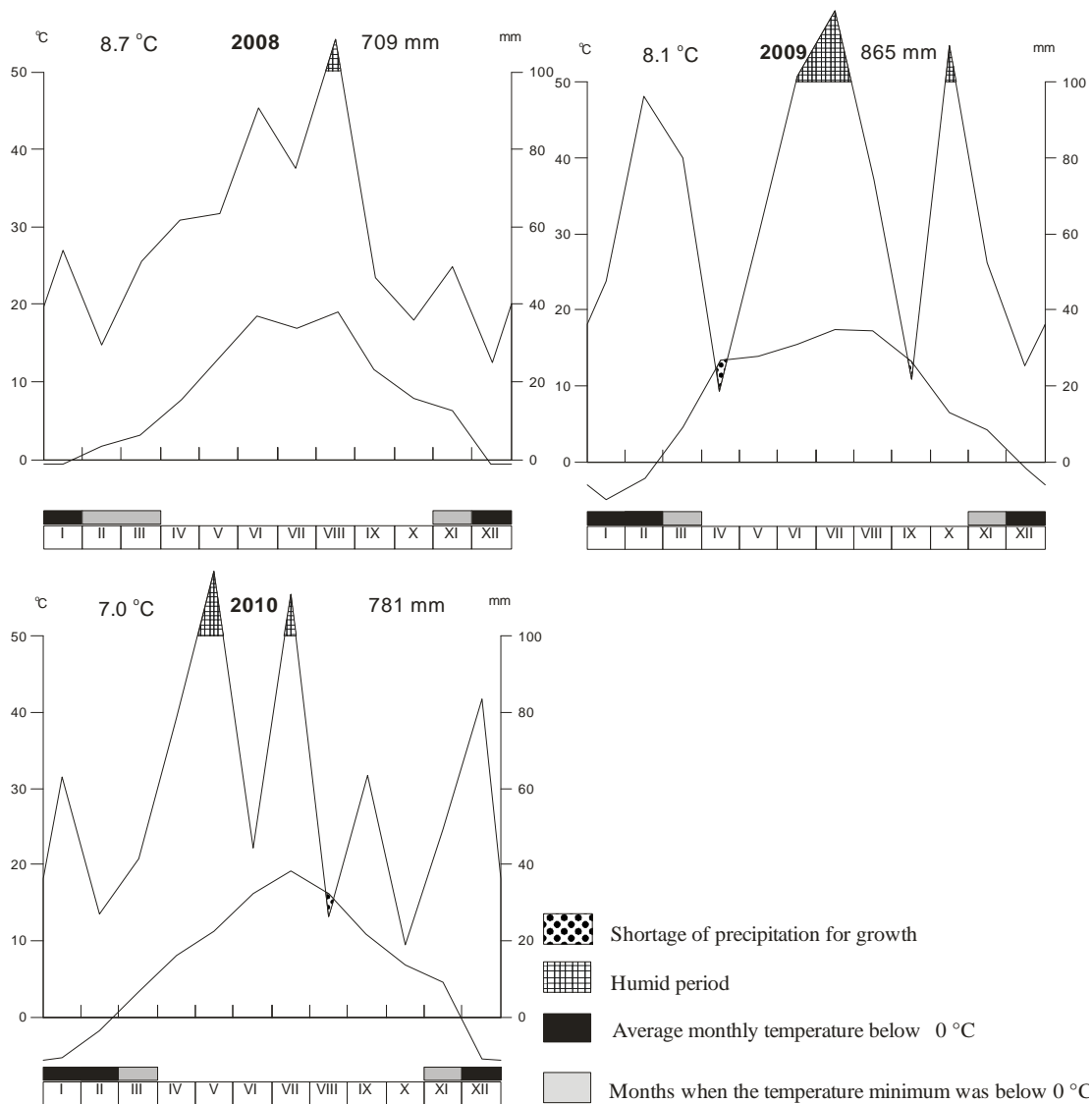
Mean values in the same column with different superscripts (a, b,c) are significant at a level of  $P < 0.05$ . R = coefficient of correlation,  $R^2$  = coefficient of determination.

results were influenced by weather conditions which were different in three years of experiment (Figures 1 and 2). The results were changed depending on weather conditions. Silages from the control stands (without additional sowing) were characterized by a higher ( $P < 0.05$ ) content of not only acetic acid ( $12.1 \text{ g kg}^{-1} \text{ DM}$ ), but also butyric acid ( $8.2 \text{ g kg}^{-1} \text{ DM}$ ). These silages showed the narrowest LA/AA ratio (4.13) and the highest pH. Silages from the experimental stands (with additional sowing) had lower contents of not only acetic acid (from  $7.0$  to  $9.6 \text{ g kg}^{-1} \text{ DM}$ ), ( $P < 0.05$ ), but also butyric acid (from  $3.0$  to  $7.4 \text{ g kg}^{-1} \text{ DM}$ ), ( $P < 0.05$ ). The low content ( $P < 0.05$ ) of acetic acid was observed in stands additionally sown by using the Horsch Exaktor and Horsch Disk technologies. Silages made from stands additionally sown by using the Horsch Disk technology showed the lowest ( $P < 0.05$ ) content of butyric acid as detected by the subsequent Fischer test (Table 2). In all experimental variants, the additional sowing resulted in a positively affected LA/AA ratio due to a markedly reduced production of acetic acid. Lactate production was increased only in the additional sowing by using the Horsch Disk and Lehner methods.

The use of additives for ensiling favourably affected the

resulting quality of the fermentation process. The additives reduced ( $P < 0.01$ ) the pH value primarily through the increased ( $P < 0.01$ ) content of lactic acid and reduced ( $P < 0.05$ ) content of butyric acid. As compared with the control silage (pH 4.98), the bacterial additive significantly reduced ( $P < 0.05$ ) pH value in the experimental silage (4.50). As compared with the control silage ( $798 \text{ mg } 100 \text{ g}^{-1}$ ), the used additives generally contributed also to a significant increase ( $P < 0.05$ ) of AWE, which corresponded with the total content of fermenting acids. The use of the bacterial preservative resulted in AWE increased ( $P < 0.05$ ) up to  $1025.9 \text{ mg } 100 \text{ g}^{-1}$ . Compared with the control silage, the LA content increase ( $P < 0.01$ ) with using the bacterial and bacterial-enzymatic additive was statistically significant with the increase being comparable for the two additives ( $57.6$ , resp.  $57.4 \text{ g kg}^{-1} \text{ DM}$ ) (Table 2).

The experimental sites and the applied additional sowing significantly affected also the content of organic nutrients and nutritive value (Table 3). The CP content was higher ( $P < 0.01$ ) in silages from the site in Bartošovice as well as the ADF content ( $P < 0.05$ ) as compared with the Helvíkovice site. On the other hand, the NDF and NEL contents were lower in Bartošovice ( $P$

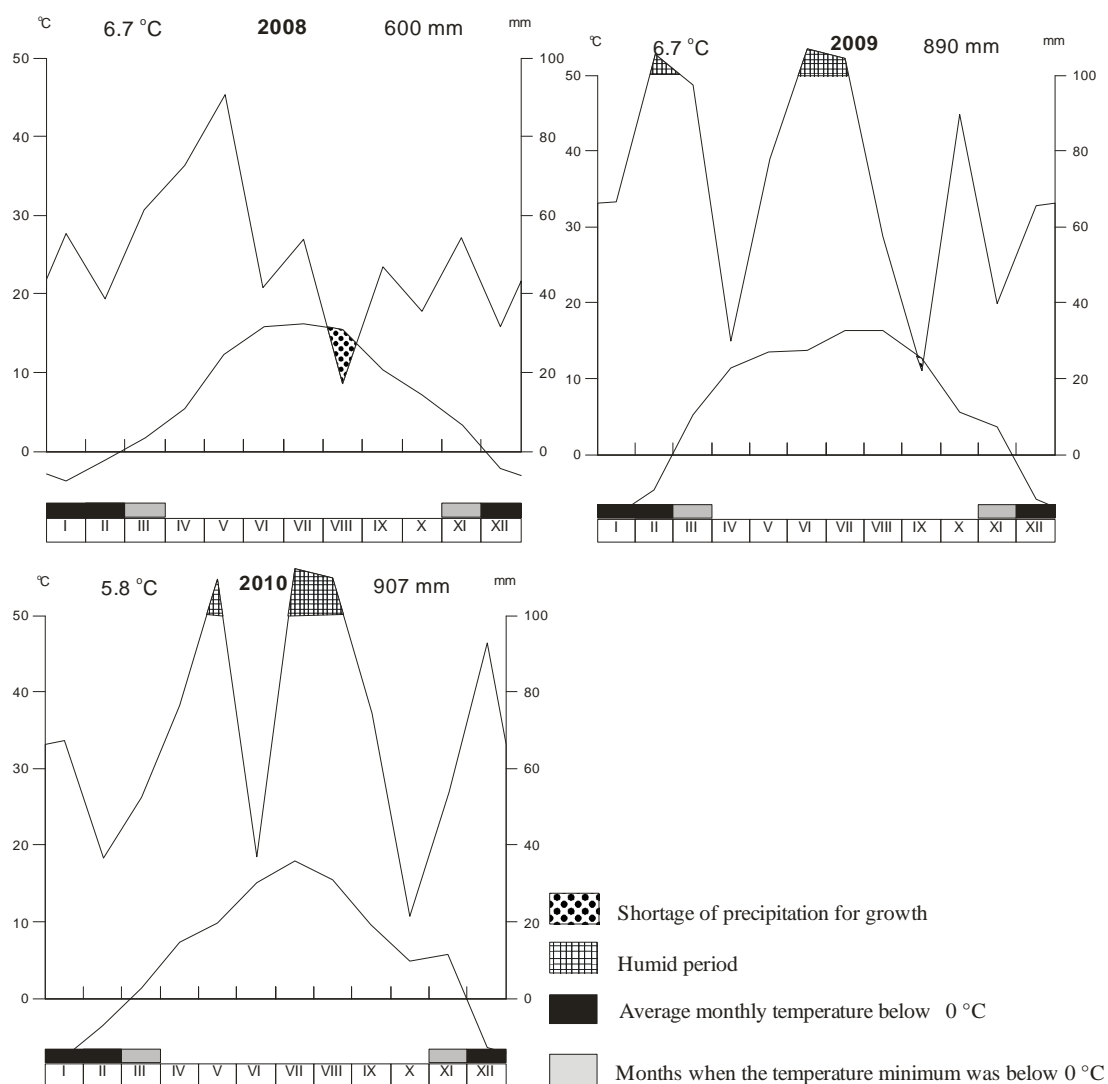


**Figure 1.** Temperature and precipitation at the experimental site Helvikovice, arranged according to Walter (1957).

< 0.01) and silages had a higher ( $P < 0.01$ ) content of ash. Additional sowing itself affected ( $P < 0.05$ ) the content of ADF fibre fraction. As compared with the control stand without additional sowing, a higher content ( $P < 0.05$ ) was detected in silages from stands additionally sown by using the Horsch Disk technology. Furthermore, the Fischer test indicated significant differences ( $P < 0.05$ ) between the stands without additional sowing and stands additionally sown in relation to the concentration of net energy for lactation (NEL). Namely silages produced from the stands additionally sown by SSD and Horsch Disk technologies exhibited a higher ( $P < 0.05$ ) NEL content ( $5.58 \text{ MJ NEL.kg}^{-1} \text{ DM}$ ). Similarly, the Fischer test pointed to differences ( $P < 0.05$ ) in the ash content (Table 3).

## DISCUSSION

The low DM content on the Bartošovice site (Table 2) resulted both from a different (lower) wilting time and a certain role might have been played also by the stand species composition with dominant clover grasses (up to 50% D), esp. white clover. The difficult wilting of white clover could have affected the resulting DM content, too. The effect of legumes can be confirmed also by the higher CP content and by the lower NEL concentration in silages from Bartošovice. The DM content of 23.3% should be considered low. The DM content recommended for ensiling legumes by the technology of wrapped bales is at least 40% (Richard and Limin, 2007), respectively higher than 35%. The higher dry matter



**Figure 2.** Temperature and precipitation at the experimental site Bartosovice, arranged according to Walter (1957).

content led to the significant reduction of the fermentation process and hence to the production of fermentation acids, which is confirmed by previous findings of other authors (Jambor and Dufková, 1993; Keller and Nonn, 1995; Weissbach, 1993). Nevertheless, in spite of the fact that the DM content was low, the pressed silages meet requirements for the nutrition of ruminants. The impact of lower dry matter in the ensiled biomass shows particularly in the increased contents of butyric and acetic acids. Dry weight of the ensiled stand affected also the pH value of resulting silages (Table 2). The lower content of dry matter in the ensiled forages points to a certain technological risk of the formation and leakage of silage fluids and also to a risk of higher pH value because a greater amount is produced of weak organic acids (acetic and butyric). A question is to what extent it would be possible to influence pH by the number of applied foil

layers. In this context we have to quote Keller and Neitz (1993) who concluded their studies with wrapped silages produced of wilted alfalfa at DM content of 35 to 40% that a good quality of wrapped alfalfa silage needs a use of six foil layers (resp. three layers with 50% overlapping). The lower content of ensiled forage is a frequent reason to higher costs connected with a higher number of foil layers and efficient ensiling additive. The authors found out that alfalfa silages wrapped in a larger number of foil layers had a lower pH value at 325 g DM/kg due to the higher production of LA and lower production of VFA. Silages with a higher DM content (> 360 g/kg) showed an opposite trend. Nonn and Keller (1993) advise as well that with the simultaneous application of ensiling additives the DM content of ensiled alfalfa should be above 30%. In our experiment with the clover-grass stand whose ensiling capacity was better than that of alfalfa, we

**Table 3.** Influence of site, technology of the additional sowing and silage additives on the content crude protein (CP), degradability crude protein (DegCP), no-degradability crude protein (NdCP), crude fiber (CF), acid-detergent fiber (ADF), neutron-detergent fiber (NDF), net energy of lactation (NEL) and ash in the years 2009 and 2010.

Parameter	CP g kg <sup>-1</sup> DM	DegCP g kg <sup>-1</sup> DM	NdCP g kg <sup>-1</sup> DM	CF g kg <sup>-1</sup> DM	ADF g kg <sup>-1</sup> DM	NDF g kg <sup>-1</sup> DM	NEL MJ kg <sup>-1</sup> DM	Ash g kg <sup>-1</sup> DM
<b>Site</b>								
Helvíkovice	136.9 <sup>a</sup>	102.7 <sup>a</sup>	34.2 <sup>a</sup>	276.3	356.6 <sup>a</sup>	561.8 <sup>a</sup>	5.66 <sup>a</sup>	66.8 <sup>a</sup>
Bartošovice	151.5 <sup>b</sup>	113.7 <sup>b</sup>	37.9 <sup>b</sup>	272.7	370.0 <sup>b</sup>	476.1 <sup>b</sup>	5.45 <sup>b</sup>	102.6 <sup>b</sup>
Level of significance	0.00001	0.00001	0.00001	0.49988	0.01618	0.00000	0.00000	0.00000
<b>Additional sowing</b>								
Control	144.9	108.7	36.2	273.6	351.6 <sup>a</sup>	514.4	5.52 <sup>a</sup>	90.1 <sup>a</sup>
Lehner	149.6	112.2	37.4	266.8	352.2 <sup>a</sup>	501.5	5.54 <sup>ab</sup>	86.1 <sup>ab</sup>
SSD	141.3	106.0	35.3	285.6	371.3 <sup>ab</sup>	535.0	5.58 <sup>b</sup>	83.0 <sup>ab</sup>
Exaktor	138.6	104.0	34.6	276.9	366.2 <sup>ab</sup>	528.3	5.56 <sup>ab</sup>	79.7 <sup>b</sup>
Disk	148.7	111.5	37.2	269.4	375.7 <sup>b</sup>	512.6	5.58 <sup>b</sup>	86.3 <sup>ab</sup>
Level of significance	0.85221	0.85247	0.85055	0.91976	0.00298	0.83369	0.13723	0.88388
<b>Additive</b>								
Control	143.1	107.3	35.8	282.3	370.7	536.6	5.55	90.7 <sup>a</sup>
Bacterial	145.0	108.7	36.2	271.5	361.3	514.5	5.56	81.1 <sup>b</sup>
Bacterial-enzymatic	146.2	109.6	36.5	271.3	359.8	508.5	5.56	83.4 <sup>b</sup>
Level of significance	0.31815	0.31776	0.31942	0.28101	0.18167	0.10998	0.74898	0.09620
<b>Year</b>								
Level of significance	0,00000	0,00000	0,00000	0,00002	0,02763	0,00003	0,68992	0,20600
R	0.7539	0.7541	0.7540	0.5767	0.5555	0.8278	0.8655	0.8918
R <sup>2</sup>	0.5683	0.5686	0.5685	0.3325	0.3086	0.6853	0.7491	0.7953

Mean values in the same column with different superscripts (a, b,c) are significant at a level of  $P < 0.05$ . R = coefficient of correlation, R<sup>2</sup> = coefficient of determination.

used for wrapping only two layers with a 50% overlap. Two layers were sufficient for production of quality silages at dry matter content below 30%. Previous studies the other way around showed that at dry matter contents below 30% (< 28 %), a risk of major bale deformation, nutrient loss and a higher risk of foil damage by breaking through occur under the effect of released fluids (Keller and Nonn, 1995; Weddell, 1995; Wilhelm and Wurm, 1999). In silages with dry matter contents below 40%, important is also the number of foil layers wrapping the bale. It was reported that, at ensiling fodder plants of lower dry matter content (less than 35% biomass), it is necessary to apply more foil layers, at least eight, when six layers are considered to be sufficient for dry matter contents of 35 to 40%. Better quality of silages wrapped in six foil layers compared with two was proved also by Forristal et al. (1999). Similarly, at ensiling forage in wrapped bales, Lingwall (2001) recommends to apply six layers of total thickness at least 0.10 mm. Keller and Neitz (1993) found the lowest ( $P < 0.05$ ) pH value ( $5.18 \pm 0.13$ ) in the silage wrapped only with three foil layers, whereas in silage bales with a

higher number of foil layers, the pH value of  $5.56 \pm 0.22$  was found. Forristal et al. (1999) found differences in pH values, comparing bales with two, four and six foil layers. Nevertheless, the two layers with a 50% overlapping in the conditions of our experiment were sufficient for the production of high-quality silage not only in respect of fermenting acids content but also in terms of nutritive value.

The additional sowing affected namely the contents of acetic and butyric acids (Table. 2). In relation to these acids, the additional sowing of *Festulolium*, oat grass, cock's foot and bird's foot trefoil is a good solution. Differences are apparent particularly in using radical technologies of additional sowing such as Horsch Exaktor and Horsch Disk. Although the employment of these technologies resulted in the highest share of additionally sown grass species in the stand (Table 1), a certain role could have been played also by sod quality. The stand without additional sowing showed a higher ( $P < 0.05$ ) ash content (Table 3) despite the fact that the same technology was used for its harvesting, which was result of the stand sod quality. The issue of cock's foot and its

influence on the silage quality may be discussed from various viewpoints. Because of the higher content of water-soluble carbohydrates, the fermentability coefficient of *Festuca* species was above that of *D. glomerata*. The proportion of lactic acid ranged from 52 to 57% and from 60 to 73% of total acids, the proportion of butyric acid ranged from 30 to 35% and from 18 to 25% for *D. glomerata* and *Festuca* species, respectively (Kaiser et al., 1999). *F. arundinacea* silages were comparable to *D. glomerata* silages in supporting milk production and superior to *D. glomerata* in terms of palatability (Fisher et al., 1993). Nevertheless, the share of cock's foot was comparable in the control and additionally sown stands with an exception of the Horsch Disk technology under the use of which it increased on the site in Helvíkovice up to 10% in 2009 (Table 1). On the other hand, exactly these stands exhibited the lowest content of butyric acid (Table 2). Rather than by the cock's foot, the variance between the control and additionally sown stands could have been caused by the greater share of bird's foot trefoil on the site in Helvíkovice, by the assertion of *Arrhenatherum elatius* and *Festulolium pabulare* (Table 1) as well as by the above mentioned sod quality. *Festulolium pabulare* could have contributed to the increased WSC content and hence to better ensiling capacity. According to Opitz (2005), the *F. pabulare* var. *Felina* exhibits a higher content of WSC than the genuine *F. arundinacea*.

The use of ensiling additives affected namely the conservation process quality. As expected, the used preparations increased the content of lactic acid and substantially suppressed the content of butyric acid (Table 2). To the contrary Baytok et al. (2005) state that percentages of butyric acids were less in the rumen fluid of sheep fed silage without additive compared with the rumen fluid of sheep fed silage treated with silage additives. In our experiment was not the content of acetic acid significantly affected by the preparations. According to Baytok et al. (2005) acetic acid concentration was the highest in silage treated with acid. The content of acetic acid could be increased by using preparations with the bacteria of *Lactobacillus buchneri*, which boost the production of acetic acid from which propylene glycol develops during a longer storage. The process improves the stability of silages. The effect on the content of nutrients was insignificant unless the content of ash is taken into account (Table 3). The positive influence of preservatives on silage quality was demonstrated. Nonn and Keller (1993), Jambor and Dufková (1993), Weddel (1995), Keller and Neitz (1993) stated a favourable effect of biological additives on the quality and stability of silages admitting at the same time that the quality of baled silage fermentation may show a considerable fluctuation. Nadeau and Buxton (1997) observed a decreased pH value and an increased LA/AA ratio in *Dactylis glomerata* silages after the application of cellulase combined with the inoculant of *Lactobacillus*

*plantarum* and *Pediococcus cerevisiae*. In our experiments, the pH of silages decreased and the LA/AA ratio increased due to the inoculant application as well (Table 2).

The hypothesis about the influence of additional sowing on the content of organic nutrients was partly confirmed. The additional sowing of drought-tolerant grass species resulted both in the increased ADF content and in the increased NEL content (Table 3). The effect of additional sowing on the content of fermenting acids was demonstrated as well. As compared with the control stands, silages from additionally sown stands contained less acetic acid and butyric acid (Table 2). The hypothesis about the use of ensiling inoculants was confirmed unambiguously. The application of the probiotic and probio-enzymatic inoculant resulted in the decreased pH, increased lactic acid content and in the minimized content of butyric acid. Nevertheless, it is impossible to confirm a zero occurrence of butyric acid in the treated silages.

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