The effects of wilting and biological and chemical additives on the fermentation process in field pea silage

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ABSTRACT: The objectives of the study were to evaluate the effects of wilting and additives on the fermentation quality of field pea silage, and to determine the rumen degradability of organic matter of pea silage. The following additives were used: commercial bacterial inoculant (1 g/t) containing homofermentative lactic acid bacteria – *Lactobacillus rhamnosus* (NCIMB 30121) and *Enterococcus faecium* (NCIMB 30122) and chemical additive containing formic acid, propionic acid, ammonium formate and benzoic acid (4 l/t). Compared to the control and chemical additive, the addition of the inoculant to wilted silage increased the lactic acid content ($P < 0.05$) and lactic:acetic ratio ($P < 0.001$). Both bacterial and chemical additives decreased ($P < 0.001$) the pH value of wilted silage. Differences between the control and chemically treated unwilted silage were also significant ($P < 0.01$). The pH value of silage with chemical additive was lower compared to the control. Proteolysis determined in wilted silage was lower compared to unwilted silage. Rumen degradability of organic matter in wilted silage treated with the chemical additive was found to be higher ($P < 0.05$) than in control and inoculant treated silages.

Keywords: field pea; fermentation quality; inoculants; chemical additive

Field pea (*Pisum sativum L.*) is an annual plant which is grown in many parts of the world. In the Czech Republic, pea is grown on 17 380 ha (Czech Statistical Office, 2008). It is highly valued for its high crude protein content. Field pea cultivation is also beneficial to improve soil fertility by the root-nodule bacteria (Rhizobia) that are able to introduce atmospheric nitrogen into soil. Field pea, which is an excellent break crop, is mainly used for seed production in the Czech Republic. However, the whole plant can be processed into silage.

Of the two main types of field pea, the one has normal leaves, whereas the other is a semi-leafless type that has modified leaflets reduced to tendrils. Dry matter degradability is similar in leafed and semi-leafless pea lines (Uzun et al., 2005).

Field pea can be ensiled as a single crop (Salawu et al., 2002) or in mixture with the whole-crop plants, for example wheat, barley and oats (Mustafa and Seguin, 2004; Pursiainen and Tuori, 2008).

Ensiling is an important method of animal feed storage for the winter season. Whittenbury (1968) coined the universal definition of silage as follows: silage is a product formed when grass or any other material of sufficiently high moisture content (and thereby liable to spoilage by microbes which thrive in the air) is stored in the absence of air.

During ensiling, soluble sugars in herbage are utilised by the microbial population to produce predominantly lactic acid, which is the main preservative agent. Furthermore, plant proteins are extensively degraded to amino acids and ammonia (McDonald et al., 1991). Because the majority of

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silage inoculants are lactic acid bacteria, these organisms are dependent on adequate amounts of fermentable water-soluble carbohydrates for growth. Low concentrations of fermentable sugars can be a problem in forage crops that have undergone excessive respiration because of the long wilting time, cloudy weather or if they were exposed to rains (Kung, 2009).

Silage quality is critical due to its effects on animal production, animal health and food quality. Additives can positively affect the quality of silage. Bacterial inoculants such as lactic acid producing bacteria are used as silage additives to enhance lactic acid production and for the better preservation of the ensiled material (Váradyová et al., 2010). Apart from biological additives, chemical additives and combinations of biological and chemical additives are used in ensiling.

Chemical additives are useful for ensiling during unsuitable climatic conditions. They mostly contain formic and propionic acids and their salts, and they are added to silage to improve aerobic stability due to their good antifungal attributes. A chemical additive can be added to the ensiled forage when its dry matter (DM) content is low, which is often encountered during rainy weather.

Degradability of silages is an important parameter of forage quality. The *in sacco* analysis, which has been applied in the present study, is the most frequently used method for determination of degradability of DM, organic matter (OM), protein, fibre, minerals and other nutrients of feeds (Jančík et al., 2009).

The evaluation of fermentation quality of wilted and unwilted silages could be complicated. Rainy weather is no exception in Central Europe, and at times conditions are unfavourable for wilting. The wilting leads to a decrease in moisture and the addition of inoculants affects the fermentation and reduces many negative effects of unacceptable fermentation (Wright et al., 2000). According to Borreani et al. (2009), wilting is applied to reduce dry matter losses in the effluent and to lower the weight of water that has to be transported from the field to the silo.

The aims of the present study were to evaluate the effects of biological and chemical additives on fermentation characteristics of unwilted and wilted field pea silages and the rumen organic matter (OM) degradability of wilted silages.

MATERIAL AND METHODS

Field pea cv. Concorde was grown in an experimental field of the Institute of Animal Science (280 m a.s.l.). The average temperature in this area in the last six years was 9.7°C with the average annual precipitation of 601 mm. Pea was planted at a seeding rate of 220 kg/ha. Whole plants were harvested at the advanced pod filling stage. At this stage over 50% of the silage was composed of pods with seeds. The first part of forage was ensiled fresh directly after harvest (approximately 23% dry matter), while the second part wilted in the swath to approximately 35% dry matter (DM) before ensiling. Both unwilted and wilted forages were chopped by a conventional forage chopper to a length of 25 mm and ensiled without any additive (C), with a biological inoculant (I), and with a chemical additive (CH). As a biological additive, a commercial bacterial inoculant was used at the amount of 1 g/t of forage. It contained the homofermentative lactic acid bacteria (LAB) *Lactobacillus rhamnosus* (NCIMB 30121) and *Enterococcus faecium* (NCIMB 30122) at amounts of 1×10^{11} CFU/g of treated forage. The inoculant (0.01 g dissolved in 0.04 l water) was sprayed onto 10 kg of fresh forage and evenly

Table 1. Nutrient content of wilted and unwilted chopped whole plants of field pea

	Unwilted (n = 8)	Wilted (n = 6)
DM (% fresh matter)	22.83	34.89
Crude protein (% DM)	14.61	15.93
Crude fibre (% DM)	29.29	29.58
Ash (% DM)	7.66	8.91
Fat (% DM)	1.87	1.11
WSC (% DM)	6.97	5.88

DM = dry matter, WSC = water-soluble carbohydrate

mingled. The control silage (C) was treated with an equivalent amount of water. The chemical additive containing formic acid (55%), propionic acid (5%), ammonium formate (24%) and benzoic acid (2.2%) was used at the amount of 4 l/t (in our case 0.04 l/10 kg of forage). Chopped forage (700 g) was packed into polyethylene bags, vacuum sealed, and stored at +18 to +20°C. Silages were analysed for fermentation quality (n = 8 for unwilted samples, n = 6 for wilted samples) after 60 days of preservation. The nutrient content of the field pea is shown in Table 1.

Silage DM was determined by oven drying at 60°C to a constant weight. The content of crude protein was determined according to the Kjehldal method, (N × 6.25) using a Kjeltec 2400 Analyser unit (FOSS Tecator AB, Höganäs, Sweden). A Fibertec™2010 system was used to analyse fibre content according to the AOAC (2005). Silage pH was measured in 100 g of fresh silage diluted in 1000 ml demineralised water with 2 ml toluene added using an InoLab pH 730 pH meter (WTW GmbH, Weilheim, Germany). Lactic acid, acetic acid, propionic acid and butyric acid were analysed according to Kvasnička (2000) by an Ionosep 2003 analyser (RECMAN – laboratory equipment, Ostrava, Czech Republic). Water-soluble carbohydrate (WSC) content was determined according to the Luff Schoorl EEC official method (AOAC, 2005), and ammonia was analysed spectrophotometrically by Libra S 22 (Biochrom Ltd., Cambridge, UK) using Nessler's reagent (AOAC, 2005).

Rumen OM degradability of wilted silages (C, n = 6; I, n = 6; CH, n = 6) was evaluated by the standard *in situ* method (Třináctý et al., 1996) in the rumen of two cannulated dry cows of the Czech Fleckvieh breed. Cows were fed twice a day a diet containing maize silage (4.8 kg DM), lucerne hay (1.8 kg DM) and a supplementary feed mixture (1.8 kg DM). Samples were weighed into nylon bags (pore size 42 µm), placed in the rumen of cows (three nylon bags/sample/cow) and incubated for 24 h. After incubation, bags were removed from the rumen and subsequently washed in cold water until the rinsing water was clear. Residues were dried at 60°C for 24 h, analysed for organic matter content, and degradability was calculated.

In addition, proteolysis was calculated as the percentage of ammonia nitrogen in total nitrogen. During proteolysis proteins are decomposed into ammonia and biogenic amine.

Dry matter losses were not measured.

Statistical analyses were performed using the GLM Procedure of SAS (SAS, 2006). The model involved the fixed effects of treatment with inoculant and wilting, and the interaction of treatment × wilting. Differences between means were evaluated by Tukey's test.

RESULTS AND DISCUSSION

The results of fermentation characteristics in unwilted and wilted silages are presented in Table 2.

The inoculant and chemical additive influenced WSC content in wilted and unwilted silages. The lowest numerical content of WSC was found for the inoculant treatment. However, differences were not significant. This numerical difference was due to a higher utilisation by the microbial population. The highest content of WSC was determined in unwilted silage treated with the chemical additive. Differences in WSC content were detected between CH and C ($P < 0.05$), CH and I ($P < 0.0001$). In wilted silage the highest content of WSC was stored in the CH treatment, and it was different ($P < 0.05$) from the content found in the I treatment.

The use of the bacterial inoculant increased ($P < 0.05$) the content of lactic acid in wilted silage. Lactic acid in unwilted I silage was higher ($P < 0.001$) than in the CH treatment, but similar ($P > 0.05$) to that in C. Borreani et al. (2005), who found out when examining pea silages made from pea harvested at four stages of growth that inoculants increased lactic acid contents in all silages except for those made from pea harvested at the end of the flowering stage. Fraser et al. (2001) reported that fermentation in pea silage was improved by the application of an inoculant containing *Lactobacillus plantarum*. The decrease in the production of lactic acid in unwilted silage caused by the chemical additive when compared to the control ($P < 0.05$) and inoculant ($P < 0.001$) treatments in the present study is in agreement with results published by Doležal et al. (2005).

The content of acetic acid was lower ($P < 0.05$) in CH compared to C and I, respectively. A chemical additive was also found by Gálik et al. (2008) to decrease the content of acetic acid compared to the control in ensiled crimped corn. The treatment had no effect ($P > 0.05$) on acetic acid contents in wilted silages. However, an interaction was present between treatment and wilting. Within treatments, wilted silage was different from un-

Table 2. Silage fermentation characteristics as affected by wilting and the addition of inoculant or chemical additive

	Treatment (T)[c]					Significance[e]		
	WS[d]	C	I	CH	SE	T	WS	T × WS
DM (%)	U	22.03	21.80	22.24	0.304	n.s.	***	n.s.
	W	32.83	33.29	33.49	0.351			
WSC (%)	U	1.44[a]	0.88[a]	2.65[b]	0.216	***	n.s.	n.s.
	W	1.30[ab]	1.06[a]	2.28[b]	0.250			
Lactic acid (%)[f]	U	2.56[a]	2.87[a]	2.02[b]	0.126	***	*	*
	W	2.30[a]	3.29[b]	2.54[a]	0.146			
Acetic acid (%)[g]	U	0.45[a]	0.51[a]	0.34[b]	0.024	n.s.	**	***
	W	0.54	0.44	0.52	0.027			
Propionic acid (%)	U	0.07	0.06	0.08	0.015	n.s.	***	n.s.
	W	0.14	0.11	0.14	0.018			
Butyric acid (%)	U	0.09	0.07	0.07	0.031	n.s.	n.s.	n.s.
	W	0.16	0.10	0.09	0.036			
LA/AA[h]	U	5.79	5.71	5.84	0.262	***	NS	***
	W	4.31[a]	7.54[b]	4.89[a]	0.302			
pH[i]	U	4.08[a]	4.02[ab]	3.94[b]	0.023	***	**	*
	W	4.31[a]	4.10[b]	4.08[b]	0.027			
Proteolysis (%)	U	1.79	1.82	1.58	0.147	n.s.	***	n.s.
	W	1.37	1.02	1.00	0.170			

C = control, I = bacterial inoculant, CH = chemical additive, SE =standard error, WS[d] = wilting status (U = unwilted, W = wilted); DM = dry matter, WSC = water-soluble carbohydrate, LA = lactic acid, AA = acetic acid

[a,b]mean values in the same row with different superscripts differ at $P < 0.05$

[e]significance of treatment: T × WS = interaction between WS and T

[f]within treatments, wilted was not different from unwilted at $P < 0.05$

[g]within treatments, wilted was different from unwilted at $P < 0.05$ in CH

[h]within treatments, wilted was different from unwilted at $P < 0.05$ in C and I

[i]within treatments, wilted was different from unwilted at $P < 0.05$ in C and CH

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, n.s. = not significant

wilted one at $P < 0.05$ in CH. Acetic acid content was higher in wilted than in unwilted silage. However, the opposite was demonstrated for I. The plausible explanation is that the lactic acid bacteria may be classified as homofermentative or heterofermentative based on their by-products of sugar fermentation. Homofermentation gives only lactic acid as the end product of glucose metabolism. In hetero-fermentation equimolar amounts of lactic acid, carbon dioxide and ethanol or acetic acid are formed from glucose via the phosphoketolase pathway. In the mixed acid fermentation ethanol, acetic acids and formate can be formed in addition to lactic acid by homofermenting LAB under certain conditions e.g. glucose limitation (Mårtensson, 2002). In the present study the added homofermentative bacteria might have utilised WSC in the wilted forage more effectively than the heterofermentative bacteria that naturally occurred in wilted forage.

Butyric acid was detected in all the silages at low amounts, with no differences ($P > 0.05$) among treatments. Contents of butyric acid were 0.16 and 0.09% for wilted C and unwilted C silage, respectively. Part of the biomass could have been contaminated by small amounts of clay during wilting in the field, resulting in undesirable clostridial fermentation. This might have been responsible for the higher variability in this group. Differences between groups were not statistically significant. The presence of butyric acid is consistent with previous findings of Borreani et al. (2009) for field pea.

Ratios of acids are important determinants of fermentation quality. The lactic (LA) to acetic (AA)

acid ratio is a good indicator of the efficiency of silage fermentation. Ideally, this ratio should not be lower than 3:1, a higher ratio between LA and AA (Jalč et al., 2009). The addition of the inoculant to wilted silage increased ($P < 0.001$) the LA/AA ratio for C compared to I, and for CH compared to I. The ratios in wilted C and CH silages were lower compared to unwilted silages. On the contrary, in the I treatment the ratio was higher in wilted than in unwilted silage. This could be attributed to the higher LA and lower AA contents in wilted silage treated with the inoculant.

Bacterial and chemical additives decreased the pH of wilted silages. Differences ($P < 0.001$) were found between C and CH ($P < 0.001$) and between C and I silages. In unwilted silages differences between C and CH were significant ($P < 0.01$). The pH of unwilted silage with the inoculant was numerically lower than that of the control treatment, but the difference was not significant. The pH of unwilted silages was lower ($P < 0.001$) than that of wilted silages for treatments C and CH, but not for I. This corresponds to results obtained by Haigh and Parker (1985). They observed that a chemical additive with formic acid significantly reduced pH, whereas wilting increased silage pH. According to Weissbach (2003) pH values below 4.2 with 200 g DM/kg and below 4.45 with 300 g DM/kg are needed to obtain well-fermented and stable silage. Results of the present study comply with these requirements.

The pH of unwilted CH silage was 3.94. However, the analysed contents of acids were low, with only 2.02% of lactic acid found (Table 2.). The pH was not related to the analysed acid contents presented only in Table 2. Unwilted CH silage was treated with the chemical additive containing formic acid, propionic acid, ammonium formate and benzoic acid. The pKa of formic acid is 3.75, and that of lactic acid 3.85. With the pKa defined as the negative common logarithm of the acid dissociation constant, these values show that formic acid is twice stronger than lactic acid. This resulted in a lower pH value in unwilted CH compared to unwilted C and I, although the contents of analysed acids in CH were lower than in C and I.

Calculated proteolysis was lower ($P < 0.001$) in wilted than in unwilted silage. This is in agreement with Cavallarin et al. (2005), who observed that the silage with DM content lower than 320 g/kg underwent butyric acid fermentation and increased proteolysis. The chemical additive positively influenced proteolysis. The CH treatment resulted in the lowest numerical value. Proteolysis is caused by undesirable microorganisms, especially clostridia. The chemical additive used in the present study contained formic acid, which appears to be one of the most effective fermentation inhibitors of clostridial growth together with hexamethylene and nitrite (Jonsson et al., 1990; Lättemäe and Lingvall, 1996).

Rumen degradability of OM measured in wilted C, I and CH silages was 57.35%, 57.56% and 61.75% (SE 0.82), respectively. The values were higher ($P < 0.05$) in CH than in C or I, with no differences ($P > 0.05$) between C and I silages. This corresponds to Succu et al. (2006), who reported that the addition of bacterial inoculants did not influence the rumen OM degradability of wheat silages.

Based on the results of the present study, the addition of both bacterial inoculant and chemical additives to unwilted and wilted field pea silages can be recommended. Generally, wilted silages showed better fermentation characteristics than unwilted ones.

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