Effect of the stage of maturity on the leaf percentage of lucerne and the effect of additives on silage characteristics

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ABSTRACT: The first part of the study concerns the effect of the stage of maturity on nutrient content and leaf percentage of lucerne (*Medicago sativa*). The plants of lucerne were harvested and analysed at several stages of growth. The crop yield and changes in the concentration of nutrients were determined. The leaf percentage from whole herbage and leaf yield were determined as well. The leaf percentage at the growth stage of small buds was significantly ($P < 0.05$) higher than at the growth stage of bloom in all three cuts (1^{st} cut 52.7% vs. 46.62%; 2^{nd} cut 52.03% vs. 44.70%; 3^{rd} cut 50.58% vs. 46.26%). Crude protein content of lucerne was decreased significantly ($P < 0.05$) from the large bud growth stage (small buds 219.6 g/kg DM and large buds 203.1 g/kg DM vs. bloom 173.5 and after bloom 154.2). In the second part of the study, the effect of silage additives on fermentation characteristics of lucerne was investigated. The bacterial inoculant (containing homo- and heterofermentative lactic acid bacteria), chemical additive (containing formic acid, propionic acid, ammonium formate and benzoic acid) and the bacterial inoculant with benzoic acid were used for the improvement of fermentation process.The addition of the inoculant with *L. buchneri* increased acetic acid ($P < 0.05$) concentration compared to the silage with chemical additive (group I with inoculant – 1.22%; group Ch with chemical additive – 0.84%; group ICh with chemical additive and inoculant – 1.43). Control silage (C) without additive contained 1.14% acetic acid in dry matter.

Keywords: lucerne; leaves; stems; buds; bloom; nutrients

Lucerne (*Medicago sativa*) accounts for a substantial part of diets for livestock. In the Czech Republic, lucerne is grown on 80 thousands ha (2.62% of arable land). Its positive attribute is a high crude protein content. The stage of lucerne maturity at harvest significantly influences the concentration of nutrients except crude protein, thus it is very important to choose a suitable date of harvesting (Yu et al., 2004). Lucerne leaves and stems contain different crude protein and crude fibre concentrations at different stages of growth. Herbage harvested at full bloom is expected to have a higher stem proportion than less mature herbage (Kilcher and Heinrichs, 1974; Fick and Holthausen, 1975). The proportion of leaves in lucerne at the time of harvest is a major factor that determines the quality of the crop (Jung, 2005).

The ensiling of lucerne is difficult due to its high buffering capacity and a low content of water-soluble carbohydrates (WSC). Thus, the addition of silage additives exerts a positive effect on the fermentation process (Gallo et al., 2002; Doležal et al., 2005).

Lactic acid bacteria (LAB) are ubiquitous in the natural environment. They ferment soluble sugars and produce lactic acid as the main fermentation

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end-product. The addition of selected LAB to silages results in faster fermentation and improves the silage quality. Apart from biological additives also chemical additives and combinations of biological and chemical additives are used in ensiling. The biological additives contain LAB or LAB with enzymes. The chemical additives mostly contain formic and propionic acid and their salts. The combined chemical-biological additives contain LAB (or LAB + enzymes) supplemented with salts of benzoic or sorbic acid.

LAB can be divided into two physiological groups: homofermentative and heterofermentative LAB. Heterofermentative LAB reduce a portion of fructose to mannitol in addition to producing CO_2, lactic acid and acetic acid when fructose is the sole carbohydrate source. On the other hand, homofermentative LAB produce 2 mol of lactic acid from any fermentable hexose, including fructose (McDonald et al., 1987).

The aim of this study was (i) to assess the effect of harvest date on the yield and quality of lucerne, and (ii) to compare the effects of additives on quality parameters of lucerne silage.

MATERIAL AND METHODS

The effect of harvest date on the yield and quality of lucerne herbage was determined in the first experiment (2004). The objective of this experiment was to examine changes in leaf and stem characteristics of lucerne herbage. These data were determined at the growth stage of small buds and bloom during the first, the second and the third cut.

Lucerne, cultivar Europe, was grown in an experimental field of the Research Institute of Animal Science (280 m above sea level) near Prague. The average temperature in this area during the last six years was 9.7°C and average total precipitation was 601.3 mm. Lucerne was planted at the seeding rate of 18 kg/ha with wheat as a foregoing crop and legume-cereal mixture as a cover crop. The area 15 × 15 m was marked out at each cut in the 10 ha lucerne field and six samples (each 1 × 1 m) were chosen for cutting at the growth stage of small buds and six samples at growth stages of large buds, bloom and after bloom. Six samples (each 1 × 1 m) were cut at two growth stages (small buds and bloom). The remaining part of the 10 ha field was cut at stages of maturity when the 30% growth had large buds. The area 15 × 15 m was marked out always at a different part of the 10 ha field. For these reasons the area for experimental cut could not be influenced by the early and late date of cut. The height of stubble was 7 cm. The herbage mass from each sample was weighed. Finally, each plant of lucerne was fractionated into leaves and stems.

The nutrient content of lucerne during the first cut was estimated as well. The nutrient content and yield of lucerne were determined at four different growth stages (small buds, large buds, bloom, after bloom). All inflorescences did not come to these stages at the same time. The respective stage is mentioned when eighty percent of inflorescences reached this stage of growth. The yield was recorded and plants were analysed.

In the second experiment (2005), four different treatments of lucerne silages were tested. Lucerne was mowed at the growth stage of small buds. The plants were after wilting on the swath to ca. 30% DM while every other row of forage was chopped by a conventional forage chopper to a length of 30 mm.

The first silage treatment was a control (without additive). In the second treatment (I) a commercial bacterial inoculant (1 g/t) containing homo- and heterofermentative lactic acid bacteria (*Lactobacillus rhamnosus*, *Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus buchneri, Pediococcus acidilactici)* was used. The chemical additive (Ch) (containing formic and propionic acid) was used in the third group of silage in an amount of 5 l/t. The preservative preparation consisted of formic acid (55%), propionic acid (5%), ammonium formate (24%) and benzoic acid (2.2%). This preservative was manufactured and delivered by Kemira Chemical Oys (Finland). The bacterial inoculant (the same as for the second group, 1 g/t) with benzoic acid in the amount of 288 g/t was used in the fourth group (ICh). All additives were applied to chopped lucerne forage at the time of ensiling.

Chopped forage (500 g) was packed into polyethylene bags. Each experimental variant was repeated seven times. After sealing in vacuum, the bags were stored at the temperature +18 to +20°C. After three months, the bags were opened and analysed.

The content of crude protein was determined according to Kjehldal method, N × 6.25. Fibre was analysed on Fibertec 2010 according to AOAC

Official Methods of Analyses 978.10 fibre in animal feed (1979); revised 2005. The pH value was measured with pH meter WTW Level 1. Lactic acid, acetic acid, propionic acid and butyric acid were analysed on Ionosep 2003. The WSC was determined according to Luff Schoorl EEC official method (79/786/EEC, Annex II).

Correlations were calculated by Excel (MS Office). The results were analysed at first by ANOVA test and in the case of some significant differences we used the *t*-test subsequently. The Tukey comparison procedure was applied to all treatments. Significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Results of the first experiment are presented in Table 1. The leaf percentage at the growth stage of small buds was significantly ($P < 0.05$) higher than at the growth stage of bloom in all three cuts. At the growth stage of small buds the leaves predominated above stems and the harvested mass was superior. At this stage of growth the leaf percentage was always higher than 50%, at the growth stage of bloom the leaf percentage was only 44.70–46.62%. At this stage of growth the crude protein content and WSC content were reduced and crude fibre was increased. During the higher growth stage of lucerne the content of crude protein decreases. Observations similar to this study were reported by Beauchemin and Iwaasa (1993).

At the growth stage of bloom the yield of whole plants of lucerne was significantly higher. This increment was realised by the growth of stems mainly. Yields of dry matter were the highest at the 1st cut and they were the lowest at the 3rd cut (Table 1). This result corresponds with Borreani and Tabacco (2002).

Table 1. Leaf percentage from whole herbage, whole herbage yield and leaf yield (in 2004)

	1st cut	SE	2nd cut	SE	3rd cut	SE
Leaves from herbage (%)						
Small buds (%)	52.07[a]	1.01	52.03[a]	1.22	50.58[a]	2.16
Bloom (%)	46.62[b]	1.95	44.70[b]	1.10	46.26[b]	1.04
Leaf yield						
Small buds (t/ha)	1.86[a]	0.23	1.23[a]	0.25	1.03[a]	0.13
Bloom (t/ha)	2.30[b]	0.32	1.55[b]	0.11	1.60[b]	0.19
Whole herbage yield						
Small buds (t/ha)	3.57[a]	1.43	2.37[a]	2.48	2.03[a]	0.65
Bloom (t/ha)	4.93[b]	2.51	3.47[b]	1.52	3.43[b]	0.93

for the same observed characteristics, mean values in the same column with different superscriptsare significantly different ($P < 0.05$)

Table 2. Nutrient content of lucerne at four stages of maturity (1st cut) in 2004

	Small buds ($n = 6$)	SE	Large buds ($n = 6$)	SE	Bloom ($n = 6$)	SE	After bloom ($n = 6$)	SE
Dry matter (g/kg DM)	165.3[a]	3.03	175.2[a]	4.61	207.8[b]	8.37	231.8[b]	1.15
Crude protein (g/kg DM)	219.6[a]	4.30	203.1[a]	4.10	173.5[b]	2.67	154.2[c]	0.53
Crude fibre (g/kg DM)	226.7[a]	4.81	235.4[a]	5.38	320.4[b]	9.08	312.7[b]	3.78
WSC (g/kg DM)	37.4[d]	1.41	45.3[c]	1.71	27.6[b]	0.69	23.3[a]	0.57
Yield of DM (t/ha)	3.6[a]	0.09	3.9[a]	0.14	4.9[c]	0.39	5.1[c]	0.05
Date of harvest	12. 5.		18. 5.		28. 5.		6. 6.	

mean values in the same line with different superscripts are significantly different ($P < 0.05$)

Table 3. Nutritive value and fermentation characteristics of lucerne silage (in 2005)

	C	SE	I	SE	Ch	SE	ICh	SE
Dry matter (g/kg DM)	317.7	7.50	313.7	5.04	313.8	4.51	310.7	4.76
Crude protein (g/kg DM)	257.8	2.15	258.7	1.64	262.0	2.64	258.5	2.24
Crude fibre (g/kg DM)	260.5	4.06	262.3	3.17	265.2	4.31	268.0	2.10
WSC (g/kg DM)	3.39	0.46	4.97	0.56	4.11	0.64	3.37	0.17
Lactic acid (%)	2.89	0.21	3.37	0.11	3.33	0.10	3.01	0.17
Acetic acid (%)	1.14	0.12	1.22^{b}	0.09	0.84^{a}	0.03	1.43^{b}	0.07
Propionic acid (%)	0.29	0.09	0.10	0.02	0.19	0.01	0.16	0.01
Butyric acid (%)	0.03	0.02	0.08	0.01	0.14	0.01	0.11	0.004
pH	4.45	0.04	4.47	0.06	4.38	0.03	4.47	0.03
KVV (mg KOH/100 g)	1 639.00	40.7	1 673.00	16.1	1 722.00	27.0	1 670.00	19.9
NH_3-N (%)	0.13^{a}	0.18	0.09^{a}	0.01	0.05^{b}	0.04	0.07	0.05
Proteolyses (%)	27.8^{a}	1.20	18.9^{b}	0.54	10.6^{d}	0.60	14.4^{c}	0.63

mean values in the same line with different superscripts are significantly different ($P < 0.05$)

C = control; I = silage with bacterial inoculants; Ch = silage with chemical additive; ICh = silage with bacterial inoculant and benzoic acid

The results of chemical analyses are shown in Table 2. The crude protein content of lucerne was decreased significantly after the large bud stage ($P < 0.05$). The highest water soluble carbohydrate (WSC) content was observed at the stage of large buds. Thereafter the WSC content decreased while the fibre crude content increased ($P < 0.05$). At the growth stage of after bloom crude protein content was the lowest and crude fibre content was the highest. The same case occurred in WSC content. It was the highest at the growth stage of large buds. After this stage WSC content was decreasing. The correlation between WSC and crude fibre was negative: –0.182. An amount of WSC is very important for the fermentative process. When the crop stand passes over to the bloom stage, the nutrient content changes and the forage quality falls. It is due to the fact that some nutrients are transferred to the generative organs. Crude protein content at the stage after bloom was reduced by about 30% in comparison with the stage of small buds.

The results of chemical analyses carried out within the second experiment are shown in Table 3. The pH of silage with chemical additive was the lowest but these differences were not significant. The lowest concentration of lactic acid was observed in the control silage. All additives increased the concentration of lactic acid. The lactic acid is also formed in silage with chemical additive, but very slowly. Because the bags with silages were opened after three months, there was time enough for the formation of a detected amount of lactic acid in silage with chemical additive. Silage I contained the highest concentration of lactic acid.

Silages treated with the inoculant that contained *L. buchneri* increased ($P < 0.05$) the acetic acid concentration compared to the silage with chemical additive. Observations similar to this study were reported by Kung et al. (2003), Nishino and Touno (2005). Acetic acid can improve the storability of silage.

The dry matter content of lucerne silage was very low due to unsuitable climatic conditions during harvest. The optimum of lucerne ensiling is 35–38%. In our case the dry matter amounted only to 31.7%. This fact apparently influenced WSC content in silage. After treatment with chemical additive the same amount of WSC was observed as in silage with biological additive.

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

The results show that the higher utilisation of WSC by the microbial population in treatment with chemical additive caused a higher content of lactic acid. However, no statistical differences were found.

Ammonia nitrogen content was also measured. The effect of lucerne silage dry matter on ammonia nitrogen content was described by Jambor (2000). He found out that the highest content of undesirable ammonia nitrogen was in silage with very low dry matter (25%). The additives (biological and chemical) positively influenced the content of ammonia nitrogen. The same case was found out in this work (Table 3). The highest content was observed after treatment without additives and the lowest content after treatment with chemical additive.

Proteolyses were also calculated. The results correspond with those of Jambor (2000). The proteolysis of the control group was the highest (27.8%). The additives positively influenced this process. The lowest proteolysis was in the group with chemical additive.

CONCLUSIONS

At the growth stage of small buds the leaf percentage was significantly higher than in the growth stage of bloom. The harvested mass was of higher quality. It can be recommended to ensile lucerne at the maturity stage of large buds. At this stage the crude protein content was only about 7.5% lower than at the small buds stage and at this stage the WSC content was highest and the crude protein content was still optimal. The yields of dry matter were highest at the maturity stage after bloom. At the stage of maturity after bloom the dry matter yield was increased but the nutritive value was reduced.

All the additives improved the quality of lucerne silage. All treated silages contained higher amounts of lactic acid. The addition of the inoculant with *L. buchneri* improved acetic acid in the silage. The additives decreased the intensity of proteolysis process.

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REFERENCES

Beauchemin K.A., Iwaasa A.D. (1993): Eating and activities of cattle fed alfalfa or orchard-grass hervested at 2 stages of maturity. Canadian Journal of Animal Science, 73, 79–88.

Borreani G., Tabacco E. (2002): Fermentation and losses of wrapped big bales of lucerne as affected by baler chopping system. In: Proc. XIIIth International Silage Conference. Auchincruive: SAC, Great Britain, 110–111.

Doležal P., Pyrochta V., Doležal J. (2005): Effects of chemical preservative and pressing of ensiled sugar-beet pulp on the quality of fermentation process. Czech Journal of Animal Science, 50, 553–560.

Fick G.W., Holthausen R.S. (1975): Significance of parts other than blades and stems in leaf-stem separations of alfalfa herbage. Crop Science, 15, 259–262.

Gallo M., Jambor V., Mlynár R., Rajčákova Ľ. (2002): Effect of application of different silage preparations upon the fermentation process in red clover. In: Proc. XIIIth International Silage Conference, Auchincruive: SAC, Great Britain, 110–111.

Jambor V. (2000): The effect of biological preservatives, treatment and different levels of dry biomass on proteolysis and content of biogenic amines in alfalfa silage. Czech Journal of Animal Science, 45, 185–188.

Jung H.G. (2005): Impact of alfalfa growth and development on forage quality traits. Asa-Cssa-Sssa Annual Meeting Abstracts. CD-Rom. Paper, 54–4.

Kilcher M.R., Heinrichs D.H. (1974): Contribution of stems and leaves to the yield and nutrient level of irrigated alfalfa at different stages of development. Canadian Journal of Plant Science, 54, 739–742.

Kung L., Taylor C.C., Lynch M.P., Neylon J.M. (2003): The effect of treating alfalfa with *Lactobacillus buchneri* 40 788 on silage fermentation, aerobic stability, and nutritive value for lactating dairy cows. Journal of Dairy Science, 86, 336–343.

McDonald L.C., McFeeters R.F., Daeschel M.A., Fleming H.P. (1987): A differential medium for enumeration of homofermentative and heterofermentative lactic acid bacteria. Applied and Environmental Microbiology, 53, 1382–1384.

McDonald P., Henderson A.R., Heron S.J.E. (1991): The Biochemistry of Silane. 2nd ed. Chalcombe Publications. Marlow Bucks, UK, 340.

Nishino N., Touno E. (2005): Ensiling characteristics and aerobic stability of direct-cut grass silages inoculated with *Lactobacillus casei* or *Lactobacillus buchneri*. Journal of the Science of Food and Agriculture, 85, 1882–1888.

Yu P., Christensen D.A., McKinnon J.J. (2004): *In situ* rumen degradation kinetics of timothy and alfalfa as affected by cultivar and stage of maturity. Canadian Journal of Animal Science, 84, 255–263.

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