Effect of legume proportion and physiological age on forage quality and the suitability of *Agrostis capillaris* L. and *Festuca rubra* L. for silage making

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ABSTRACT

The chemical and physical properties of herbage from low-input grassland usually differ from plant material of intensively managed swards. The experiment in split-plot design with four replicates was carried out to examine, whether legume proportions and the physiological age of herbage are relevant to fermentability of herbage. *Lotus corniculatus* did not increase nitrate concentrations sufficiently in *Festuca rubra* and most *Agrostis capillaris* swards to be able to avoid clostridial fermentation. However, *Lotus corniculatus* reduced the WSC/BC ratio of the grasses grown in mixture and that of the complete herbage of the mixtures. A late first cut in summer had an unexpected positive effect on the WSC/BC ratio of the grasses in pure stands, though reduced nutritive value of silages made from plants of advanced maturity stages are in all probability because of low energy concentrations in the fresh matter even with high yield proportions of the legume. Absence of fertilisation and late utilisation do not affect the chemical requirements for the fermentation process of grass-rich herbage in principle. However, silage making under these conditions is very demanding concerning ensilage technique: it may be necessary to use nitrate additives and higher fibre contents makes it more difficult to compact herbage.

Keywords: low-input grassland; legumes; fermentability; forage quality

Less intensively managed grassland gains in importance in large parts of Europe. On the one hand, this is for economical reasons, on the other hand, a multitude of nature protection programs forbid use of fertiliser and provide to delay the first cutting date.

Especially on low input conditions, in regions with insufficient productivity of pastures during the cold season, there is a need for effective conservation methods to ensure a cheap winter feeding. Compared with haymaking, silage making is advantageous because of its low costs of work, assuming the farm possesses the specific equipment. Compared with herbage of intensively managed swards, herbage grown in low-input conditions contains a high number of less productive species (Common et al. 1991), as a result of the low soil nitrogen level. As a consequence, the chemical and physical features are different. The differences increase when the use of the primary growth is delayed, e.g. for nature protection reasons. The limited forage quality of such swards and grass species concerned is well known (Haggar 1976, Armstrong et al. 1986, Frame 1991). However, there is little information on the fermentability of typical species of low-input grassland. *Agrostis capillaris* and *Festuca rubra* are dominant grass species of extensively managed pastures on medium-moist soils with relatively low pH. Since these grass species never grow up alone in these sites, it is possible that their chemical composition can also be influenced by competition. Competition for light, water and nutrients and nitrogen transfer from legumes to grass are some possible effects. Because there is no fertilisation on extensive grassland in many cases, the nitrogen fixation of the legumes is frequently the most important N-source at these sites. On intensively managed grassland the intensity of fertilisation influences the fermentability, particularly, the buffering capacity and concentrations of water-soluble carbohydrates (Jones et al. 1961, Opitz v. Boberfeld and Jucken 1995, Keady and O'Kiely 1996, 1998). Therefore, the legume proportion could have a similar effect on fermentability (Opitz v. Boberfeld and Jucken 1995). Anyway, nitrate in plants increases with an increasing rate of fertiliser-N (Shiel et al. 1999) and a low soil nitrogen level may lead to low nitrate concentrations in grass. A minimum of approximately 0.05% nitrate in herbage dry matter is required to hinder butyric acid fermentation through inhibition of clostridia increase (Weißbach and Honig 1996). Shiel et al. (1999) reported that the nitrate content of herbage *Lolium perenne*/*Trifolium repens* swards was greater than that of pure *Lolium perenne* swards receiving the same amount of fertiliser-N, even when no nitrogen was applied. Nevertheless, Opitz v. Boberfeld and Sterzenbach (2001) gener-

Table 1. Meteorological data

	Year 1		Year 2	
	mm	°C	mm	°C
January	1.6	–2.3	18.0	–4.1
February	48.2	–0.8	76.9	4.3
March	31.9	2.7	20.1	6.8
April	13.5	8.6	19.5	5.4
May	98.7	11.8	53.2	10.9
June	27.3	15.9	86.2	14.3
July	81.3	16.3	41.4	16.0
August	68.8	17.2	14.0	17.2
September	47.7	11.5	14.2	11.7
October	89.0	9.4	88.5	7.0
November	72.4	5.5	64.2	4.3
December	34.7	–1.6	57.7	2.8
Annual	615.0	7.8	554.0	8.1

ally found insufficient nitrate concentrations in more than hundred different extensively managed grassland swards, irrespectively of the botanical composition. Delaying the harvest of the primary growth has also an effect on the fermentability of herbage. Keady and O'Kiely (1998) showed that delaying the cutting date decreased concentrations of water-soluble carbohydrates and buffering capacity, whereas nitrate concentrations were not altered. Silage made from late cut primary growth of a predominantly *Lolium perenne* sward had lower dry matter digestibility, crude protein concentration ammonia N, acetate, propionate and ethanol concentrations, but no significant effect of harvest date on lactate and butyrate was detectable in this study.

The purpose of this study was to evaluate the importance of species, cutting date, legumes, and competition effects to fermentability of herbage from extensively managed swards on standardised conditions. The results should help to evaluate the risk of clostridial fermentation of herbage grown on extensively managed grassland as a basic for further studies.

MATERIAL AND METHODS

Sward management. The experiment was established on an eroded stagnic Luvisol of loess (pH 6.2) near Giessen in Central Germany, located at a height of 160 m above sea level. Meteorological data are shown in Table 1. The experiment was a split-plot design with four replications. In the seeding year and in the following two years of investigation the plots stayed unfertilised. Cutting management was the main factor (two treatments, see extra sub section cutting management) and the sub factors were grass species and proportion of *Lotus corniculatus*. The grass species were *Agrostis capillaris* and *Festuca rubra*. The proportions of *Lotus corniculatus* were 0, 25, 50 and 100%. In the mixtures, *Agrostis capillaris*, *Festuca rubra* and *Lotus corniculatus* were sown in separate seed rows according to their proportions in the mixture, so the mixture ratio 25/75, meant a ratio from one row of the legume to three rows of one of the grass species and the mixture ratio 50/50 meant alternate seed rows of grass and legume. The row distance was 17 cm in 2.8 × 5 m plots. To minimise cultivar effects, each of the species was sown as a mixture of two cultivars of different growth type; *Festuca rubra* was sown as a mixture of the subspecies *Festuca rubra rubra* (cv. NFG-Th. Roemer) and *Festuca rubra commutata* (cv. Odra). The *Agrostis capillaris* cultivars were Litenta and Tendenz. *Lotus corniculatus* was a mixture of cultivars Hoki and Odenwaelder. The species in the mixtures were harvested separately with grass shears and a 5 cm distance bar by cutting one metre each of three different rows of the species concerned. Afterwards, the remaining herbage was harvested with a Haldrup® small-plot harvester.

Cutting management. There were four harvest dates to obtain physiologically young herbage (end of May/early June, early July, mid of August, mid of October) and two harvest dates to obtain

Table 2. Growth stages at cutting dates

	Year 1		Year 2	
Physiological age species	young	old	young	old
Agrostis capillaris	prebloom stage	full flowering	flowering starts	end of flowering
Festuca rubra	stem elongation	wax-ripe stage	stem elongation	wax-ripe stage
Lotus corniculatus	prebloom stage	end of flowering, first involucres	flowering starts	involucres

Table 3. Dry matter-yield (= two subsequent cuts for young herbage, one delayed cut for old herbage)

Yield (t/ha)	Proportion of *Lotus corniculatus*	Year 1		Year 2	
		1st cut 28 May + 2nd cut 2 July	1st cut 2 July	1st cut 2 June + 2nd cut 8 July	1st cut 8 July
Agrostis capillaris	0	3.3	5.8	3.6	3.5
Agrostis capillaris	25	4.5	6.5	6.2	7.6
Agrostis capillaris	50	5.6	7.3	8.2	7.5
Festuca rubra	0	2.5	3.9	2.3	3.2
Festuca rubra	25	3.7	6.9	5.0	7.8
Festuca rubra	50	4.7	7.3	6.8	7.5
None	100	4.6	6.6	5.9	6.6
LSD (cutting management)		1.28	1.28	0.79	0.79
LSD (grass species + legume)		0.77	0.77	0.60	0.60

physiologically old herbage (early July, mid of October). Subsequently, only data from July will be presented. The late first cut in July was chosen to simulate usual nature protection conditions in Germany. To avoid interactions in WSC concentrations by different weather conditions at harvest date (Alberda 1965) data of the harvest of the primary growth in July (physiological old herbage) were compared with the primary regrowth of the four cut treatment, harvested at the same day (physiological young herbage), instead of the primary growth in May/June. The growth stages of the three species in July are shown in Table 2. The following cuts were carried out to simulate grazing, since dry matter yields were insufficient for silage making.

Analysis. The potential fermentability of the herbage was characterised by the determination of the ratio of water-soluble carbohydrate concentration to buffering capacity (WSC/BC ratio). The nitrate concentration (g NO_3/kg) was measured to predict the risk of butyric acid fermentation. The fermentation success was assessed to be good when the WSC/BC ratio was > 2, assuming wilted silage with dry matter (d.m.) concentration of 30%, and when the nitrate concentration was > 0.5 g NO_3/kg dry matter (Weißbach and Honig 1996). Concentration of WSC (%) was measured by the anthrone method (Yemm and Willis 1954) using a Milton Roy Spectronic 601 photospectrometre to determine the colouring. Buffering capacity (g lactic acid per 100 g d.m.) was determined according to the method used by Weißbach (1967) using a Metrohm 702 SM Titrino. The nitrate concentration of silage crops was measured by the Maastricht Xylenol method (Anonymus 1997) using a Milton Roy Spectronic 601, as well. In preceding analyses (Laser 1999, Opitz v. Boberfeld and Laser 1999), metabolizable energy (MJ ME/kg d.m.) was estimated *in-vitro* with rumen liquid and crude protein, ADL and yield were determined (Anonymus 1997). All results were examined by analysis of variance; where responses were significant at $P < 0.05$, least-significant differences (*LSD*) were calculated separately for main factor (cutting management) and sub-factors (grass species + proportion of *Lotus corniculatus*).

RESULTS

Dry matter yield. The dry matter yields until early July are summarised in Table 3 and take account the herbage harvested first at this time and the sum of primary growth harvested end of May/early June and re-growth at early July. The delayed harvest of the primary growth resulted in significantly higher yields in both years. Cutting management was the most important source of variation in year 1, whereas the effect of *Lotus corniculatus* proportion was more distinct in year 2. *Festuca rubra* yielded more in primary growths, but yield of the re-growth was inferior to *Agrostis capillaris*.

Herbage quality. Delaying the harvest of the primary growth resulted in decreased metabolizable energy (MJ ME/kg d.m.), presented in Table 4, increased ADF concentrations (Table 5) and decreased crude protein concentrations (Table 6). Differences in ME between the grasses in pure stands and the mixtures with *Lotus corniculatus* were small, whereas crude protein concentration clearly increased with increasing legume propor-

Table 4. Energy concentration in herbage (= 2nd cut for young herbage, 1st cut for old herbage, both harvested at the same day)

MJ ME/kg d.m.	Proportion of *Lotus corniculatus*	Year 1		Year 2	
		physiological young	physiological old	physiological young	physiological old
Agrostis capillaris	0	8.7	8.0	8.2	7.7
Agrostis capillaris	25	8.8	8.1	8.7	7.9
Agrostis capillaris	50	9.0	8.1	8.8	7.6
Festuca rubra	0	9.2	7.1	8.9	7.1
Festuca rubra	25	9.3	7.8	8.9	7.5
Festuca rubra	50	9.3	7.5	8.8	7.3
None	100	9.1	8.6	8.9	8.2
LSD (cutting management)		0.6	0.6	0.4	0.4
LSD (grass species + legume)		0.4	0.4	0.4	0.4

tion. Grass in swards with the legume had significant higher crude protein concentration than grass in monoculture.

WSC/BC ratio. Compared with the grasses in pure stands, the WSC concentration of mixtures and *Lotus corniculatus* in pure stands were significantly lower (Table 7), whereas BC increased (data not shown). The late harvest of primary growth resulted in increased WSC concentrations of *Agrostis capillaris* and *Lotus corniculatus*. WSC concentrations of *Festuca rubra* from pure stands decreased, but BC of herbage of this grass also decreased. Therefore, the WSC/BC ratio did not alter in year 1 and was even higher in physiological old *Festuca rubra* herbage in year 2 (Figure 1). Concerning the WSC/BC ratio both grass species in pure stands fulfilled the requirements for a good fermentation, though the WSC/BC ratio of *Agrostis capillaris* in monoculture after the short growing interval was relatively low in year 1, because of low WSC concentrations (Table 7). WSC/BC ratios of the mixtures and *Lotus corniculatus* in pure stands were clearly too low to ensure a sufficient acidification; the effect of *Lotus corniculatus* proportion was the most important source of variation in both harvest years. This effect was similar when the two grass species were examined as separate units (Figure 2); the WSC/BC ratio of the grasses grown in mixtures were always lower than in pure stands. Especially after short growing intervals, the WSC concentrations of the grass species decreased drastically in mixtures with *Lotus corniculatus* in both years. In

Table 5. Acid detergent Fibre (= ADF) concentration in herbage (= 2nd cut for young herbage, 1st cut for old herbage, both harvested at the same day)

ADF (% of d.m.)	Proportion of *Lotus corniculatus*	Year 1		Year 2	
		physiological young	physiological old	physiological young	physiological old
Agrostis capillaris	0	35.9	41.0	34.3	36.8
Agrostis capillaris	25	31.0	41.4	29.1	41.0
Agrostis capillaris	50	27.9	39.9	29.4	42.9
Festuca rubra	0	31.4	40.8	33.7	41.7
Festuca rubra	25	27.5	41.7	30.3	43.2
Festuca rubra	50	29.0	41.0	28.9	44.2
None	100	27.4	39.5	27.0	40.0
LSD (cutting management)		3.0	3.0	2.1	2.1
LSD (grass species + legume)		1.2	1.2	1.1	1.1

Table 6. Crude protein concentration in herbage (= 2nd cut for young herbage, 1st cut for old herbage, both harvested at the same day)

Crude protein (% of d.m.)	Proportion of *Lotus corniculatus*	Year 1		Year 2	
		physiological young	physiological old	physiological young	physiological old
Agrostis capillaris	0	10.3	7.0	9.8	6.0
Agrostis capillaris	25	12.6	11.7	12.6	10.9
Agrostis capillaris	50	16.1	13.1	22.0	11.3
Festuca rubra	0	12.0	3.9	10.1	3.7
Festuca rubra	25	13.3	6.4	14.0	8.6
Festuca rubra	50	14.2	7.6	17.2	10.5
LSD (cutting management)		2.4	2.4	3.7	3.7
LSD (grass species + legume)		0.9	0.9	2.1	2.1

year 2 WSC concentrations were reduced to less than 3% in dry matter of *Agrostis capillaris* and to less than 4% in dry matter of *Festuca rubra,* when the legume proportion was 50%.

Nitrate concentrations. As shown in Figure 3, *Agrostis capillaris* and *Festuca rubra* in pure stands were nearly nitrate-free in contrast to *Lotus corniculatus*. *Lotus corniculatus* also increased nitrate concentrations of the grass component in the mixtures. However, most concentrations remained below 0.5 g NO_3/kg d.m., in spite of high legume proportions. The exception was *Agrostis capillaris* grown in mixture with 50% *Lotus corniculatus* after longer growing intervals and year 2 after short growing intervals as well.

DISCUSSION

The limited forage value of secondary grasses, such as *Agrostis capillaris* and *Festuca rubra*, compared with *Lolium perenne* and herbage from extensively managed grassland in general is described in literature (Haggar 1976, Armstrong et al. 1986, Common et al. 1991, Frame 1991). In addition, a delayed cut of the primary growth affects forage quality of fresh herbage (Table 3). Especially the nutritive value of *Festuca rubra* decreases rapidly with advancing maturity, as also reported by Haggar (1976). Therefore, silage made from older crops are only suitable for low-input animal production systems. Decreasing fibre concentrations

Table 7. Concentrations of water soluble carbohydrates (= two subsequent cuts for young herbage, one delayed cut for old herbage)

Water soluble carbohydrates (% of d.m.)	Proportion of *Lotus corniculatus*	Year 1		Year 2	
		physiological young	physiological old	physiological young	physiological old
Grass species	1.9 0.6				
Agrostis capillaris	0	7.5	10.4	9.5	12.4
Agrostis capillaris	25	6.0	8.3	7.0	6.3
Agrostis capillaris	50	5.2	7.9	5.4	5.4
Festuca rubra	0	14.4	9.2	13.7	11.5
Festuca rubra	25	6.5	7.7	7.1	5.4
Festuca rubra	50	5.9	7.6	6.2	5.1
None	100	5.3	7.3	5.9	5.3
LSD (cutting management)		1.9	1.9	1.3	1.3
LSD (grass species + legume)		0.6	0.6	0.5	0.5

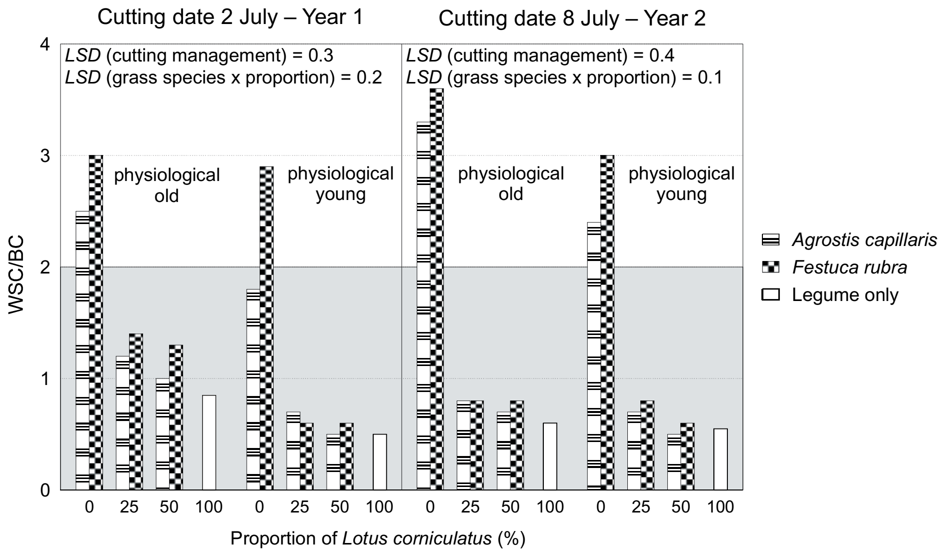

Figure 1. Effect of grass species, maturity, and proportion of *Lotus corniculatus* on the WSC/BC ratio in whole plots

make it more difficult to compact the herbage, therefore, there is a higher risk of aerobic decomposition. Furthermore, there is a higher risk of poor fermentation caused by infection with clostridia for over-ripe, soiled herbage. Increased yield is a positive effect of delaying the first harvest. Before accepting the specific difficulties when ensiling herbage from extensively managed grassland, it is imperative to know the potential fermentability of the plant material. The main aim of this study was to evaluate the meaning of factors in low-input systems for chemical pre-conditions in *Agrostis capillaris* and *Festuca rubra* that are relevant for later fermentation.

Present investigations suggest, that delaying the cut of the primary growth does not reduce the WSC/BC ratio of *Agrostis capillaris* and *Festuca rubra*, because apparently WSC concentrations increase and BC decreases with longer cutting intervals. Especially the WSC/BC ratio of young *Agrostis capillaris* is clearly worse than after long growing intervals.

Grasses without nitrogen fertilisation have higher WSC concentrations and lower BC than fertilised grass (Jones et al. 1961, Opitz v. Boberfeld and Jucken 1995, Keady and O'Kiely 1996, 1998). It is not surprising, that the WSC/BC ratio of *Agrostis capillaris* and *Festuca rubra* in pure stands met the requirements to support successful fermentation, since nitrogen was limited in these swards. Apparently, the effect of nitrogen fixation by *Lotus corniculatus* is similar to the effect of nitrogen fer-

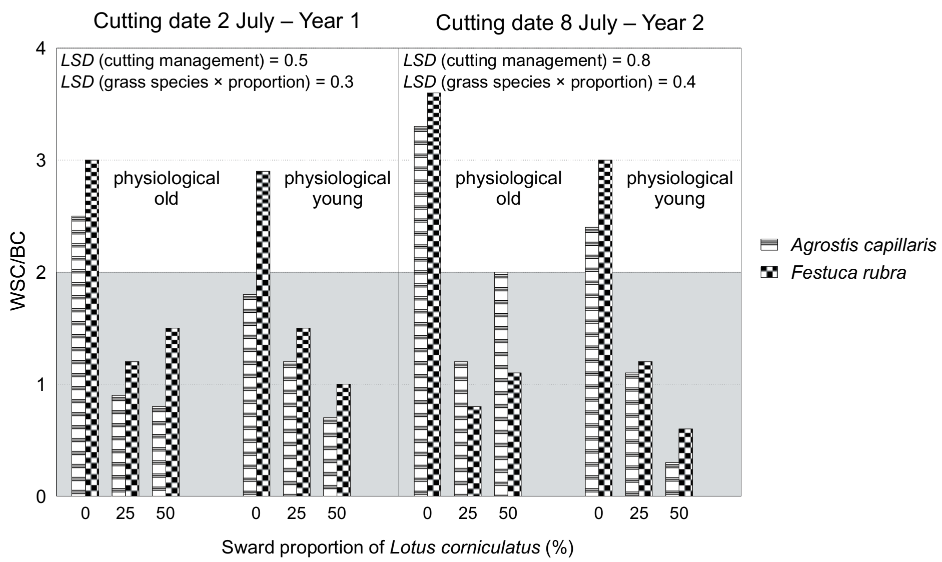

Figure 2. Effect of grass species, maturity, and proportion of *Lotus corniculatus* on the WSC/BC ratio in the grass fraction

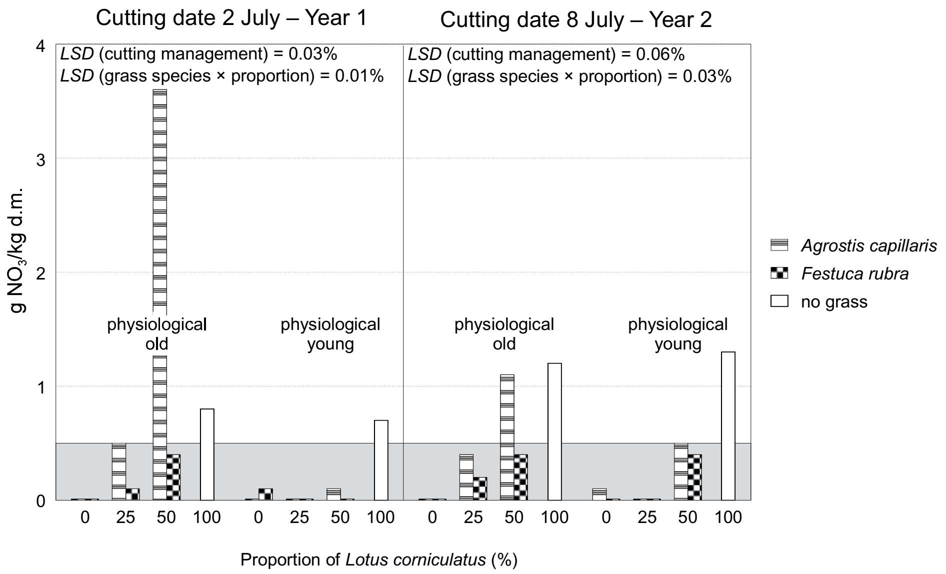

Figure 3. Effect of grass species, maturity, and proportion of *Lotus corniculatus* on the nitrate concentrations in herbage

tilisation. WSC/BC ratio of the grasses in the mixtures and of the complete herbage of the mixtures decreased drastically, compared with WSC/BC ratio of the grass species in pure stands. On the one hand, decrease of the WSC/BC ratio in mixtures is a consequence of low WSC concentrations and a high BC in the legume, on the other hand, there may be a nitrogen transfer from *Lotus corniculatus* to grass (Mallarino et al. 1990). *Agrostis capillaris* and *Festuca rubra* in mixture had higher crude protein concentrations and relative yield of grass next to *Lotus corniculatus* increased, compared with grass in pure stands. The difference in calculated nitrogen off-take by grass in mixed swards and grass in monoculture was 23–56 kg N/ha/year (Laser 1999). Dubach and Russelle (1994) confirm the conclusion, that there are several possible reasons for increasing nitrogen availability for grass in mixture with legumes: release of fixed nitrogen by decomposing root and nodule tissue, harvest residues and stubble, and reduced competition for nitrogen between legume and grass, since legumes are not as dependent on soil nitrogen as grasses. The effect of *Lotus corniculatus* on WSC/BC ratio is quite similar for *Agrostis capillaris* and for *Festuca rubra* and reduced fermentability of herbage in grass/legume swards also described for other legumes and grass species, such as *Lolium multiflorum* or *Dactylis glomerata* in mixture with *Trifolium pratense* (Opitz v. Boberfeld and Jucken 1995).

However, the nitrate concentrations remained insufficient in most cases, in spite of the high legume proportions. The nitrate concentration should be 0.5 g NO_3/kg d.m. or higher to reduce clostridial fermentation. Neither *Agrostis capillaris* nor *Festuca rubra* fulfilled this requirement in pure stands, apparently, caused by the absence of N fertilisation. The effect of *Lotus corniculatus* was significant, however, even high proportions of in the mixtures were insufficient to improve the situation of nitrate shortage in grass by additional N from nitrogen fixation in most cases. Probably nitrate was rapidly metabolised into organic nitrogen compounds. An exception was *Agrostis capillaris* after long growing intervals with 50% of *Lotus corniculatus* in the mixture. The reasons for the high nitrate concentration in these plots are unclear. Shading of the grasses by the legume is a possible explanation. Reduced light intensity is an inhibiting factor on the nitrogenase activity. The nitrogenase activity also decreases during mature (Darwinkel 1976). Both factors are known to increase nitrate accumulation in plants (Wright and Davidson 1964). Apparently, shading by the legume was meaningless for the nitrate concentrations of *Festuca rubra*, because of the more rapid development and the higher growth compared with *Agrostis capillaris*, and was also irrelevant for the secondary growth of *Agrostis capillaris*. The re-growth had lower nitrate concentrations, probably because of lower stem proportions. Stems usually contain more nitrate than leaves (Alberda 1965, Darwinkel 1976).

Shiel et al. (1999) point out that nitrate concentrations of herbage from *Lolium perenne*/*Trifolium repens* swards are greater than that of *Lolium perenne* swards. However, most nitrate concentrations of unfertilised grass/legume swards were also below 0.5 g/kg d.m.) in this experiment, in spite of proportions up to 68% of white clover dry matter in the total dry matter. Therefore, it is unlikely, that sufficient nitrate concentrations can be obtained by legumes alone. To start out from legume proportions far below 50% in typical meadows and pastures, there is actually no relevant effect of the legume on nitrate concentrations of grass and complete herbage of extensively managed grassland. However, Opitz v. Boberfeld and Sterzenbach (2001) show, that the wide-spread problem of butyric acid production in silages from low-input grassland can be solved easily by the use of nitrate additives.

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ABSTRAKT

Vliv podílu jeteloviny a fyziologického stáří na kvalitu píce a vhodnost *Agrostis capillaris* L. a *Festuca rubra* L. pro silážování

Chemické a fyzikální vlastnosti píce z low-input travních porostů se obvykle liší od píce z intenzivně obhospodařovaných porostů. Pokus byl založen metodou dělených dílců se čtyřmi opakováními s cílem zjistit, zda podíl jeteloviny a fyziologické stáří píce mají závažný vliv na její fermentaci. *Lotus corniculatus* nezvýšil dostatečně koncentraci dusičnanů ve *Festuca rubra* a ve většině porostů *Agrostis capillaris* k tomu, aby vyloučil klostridiální fermentaci. Avšak *Lotus corniculatus* snižoval poměr vodorozpustné sacharidy/pufrovací kapacita (WSC/BC) v travách pěstovaných ve směsi a v píci ze smíšených porostů. Pozdní první seč v létě měla nečekaně pozitivní vliv na poměr WSC/BC v travách z čistých porostů, ačkoliv snížená výživná hodnota siláží připravených z rostlin v pokročilých fázích zralosti je velice pravděpodobná následkem nízkých koncentrací energie v čerstvé hmotě, dokonce i při vysokém podílu jeteloviny. Vynechání hnojení a pozdní sklizeň v zásadě neovlivnily chemické požadavky na fermentační proces v píci s převahou trav. Příprava siláže v těchto podmínkách je však velice náročná na techniku silážování: bývá nezbytné použít nitrátová aditiva a vyšší obsahy vlákniny činí dusání píce obtížnějším.

Klíčová slova: low-input travní porost; jeteloviny; fermentační schopnost; kvalita píce

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