

EFFECT OF GROWTH STAGE OF LEGUMES ON SILAGE DIGESTIBILITY

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Abstract. Legumes are known to be difficult to ensile because they are highly buffering, have a low sugar concentration and often a low dry matter concentration. The previous results have shown that fermentation quality can easily be improved by using additives.

The chemical composition, nutritive value and digestibility of silage prepared from the early-maturing red clover (*Trifolium pratense* L. *subvar. praecox* Witte) varieties 'Varte' (4x) and 'Jõgeva 433' (2x); the late-maturing red clover (*Trifolium pratense* L. *subvar. serotinum* Witte) varieties 'Ilte' (4x) and 'Jõgeva 205' (2x); hybrid lucerne (*Medicago varia* Mart) varieties 'Karlu' and 'Jõgeva 118' in different phases of development were studied in 2000 and 2001. For this purpose, test silages were prepared. Legumes were cut at the height of 5 cm, chopped into 2 cm pieces, supplemented with additive AIV 2000 and conserved into jars. In 90 days the jars were opened; the chemical composition of test silages was determined according to the generally accepted methods, and nutritive value was calculated. The dry matter degradability of silages, as well as organic matter digestibility, was determined *in vitro*, using ANKOM analysers.

Comparison of the chemical composition and nutritive value of different varieties of red clover and hybrid lucerne bred in Estonia revealed the following results. At the bud formation stage, the crude protein content of the tetraploid red clover variety 'Varte' was considerably higher (197 g/kg), and that of the crude fiber was lower (208 g/kg), compared with other varieties. Due to the extremely low dry matter content 122 to 130 g/kg at the bud formation and 145 to 148 g/kg at the stage of early flowering, the tetraploid red clovers should be wilted for ensiling.

The metabolizable energy content of silage of the red clover and hybrid lucerne varieties was from 9.1 to 10.1 MJ/kg, respectively, of the stage of growth, and the organic matter digestibility - from 57 to 71 %.

Keywords: legume, red clover, lucerne, silage, digestibility, nutritive value

ANKŠTINIŲ AUGALŲ AUGIMO STADIJOS ĮTAKA SILOSO VIRŠKINAMUMUI

Santrauka. Yra žinoma, kad ankštinius augalus sunku silosuoti, kadangi jie pasižymi stipriomis buferinėmis savybėmis, maža cukraus koncentracija ir dažnai mažu sausųjų medžiagų kiekiu. Ankstyvesni tyrimai patvirtino, kad fermentacijos kokybę galima pagerinti, naudojant priedus.

2000-2001 m.m. Laikotarpiu buvo tiriama siloso, pagaminto iš anksti bręstančių raudonųjų dobilų (*Trifolium pratense* l *subvar. Praecox* witte) "Varte" (4x) ir "Jogeva 433" (2x) atmainų, ir iš vėlai bręstančių raudonųjų dobilų (*Trifolium pratense* l *subvar. Serotinum* witte) "Ilte" (4x) ir "Jogeva 205" (2x), ir liucernos hibridų (*Medicago varia* mart) "Karlu" ir "Jogeva 118" atmainų cheminė sudėtis, maistinė vertė ir virškinamumas skirtingose vystymosi stadijose. Šiam tikslui buvo paruošti siloso mėginiai. Ankštiniai buvo nupjauti 5 cm aukštyje, supjaustyti 2 cm ilgio gabalėliais ir užkonservuoti stiklainiuose, panaudojant priedą „AIV 2000“. Po 90 dienų stiklainiai buvo atidaryti, siloso mėginių cheminė sudėtis buvo tiriama įprastais metodais, be to buvo apskaičiuota maistinė vertė. Sausosios medžiagos skaidymas ir siloso organinės medžiagos virškinamumas buvo nustatyti *in vitro*, panaudojant ANKOM analizatorių.

Įvairių raudonųjų dobilų ir liucernos hibridų atmainų, išvestų Estijoje, cheminės sudėties ir maistinės vertės palyginimas, parodė, kad pumpurų formavimosi stadijoje žaliųjų proteinų kiekis tetraploidinių raudonųjų dobilų atmainoje "Varte" buvo žymiai didesnis (197 g/kg), o žaliosios ląstelienos kiekis mažesnis (208 g/kg), negu kitose atmainose. Dėl ypač mažo sausosios medžiagos kiekio, 122 - 130 g/kg pumpurų formavimosi stadijoje ir 145 - 148 g/kg ankstyvo žydėjimo stadijoje tetraploidiniai raudonieji dobilai prieš silosavimą turėtų būti vytinami.

Apykaitinės energijos kiekis raudonųjų dobilų ir liucernos hibridų atmainose augimo stadijoje buvo atitinkamai 9,1 - 10,1 mj/kg, o organinės medžiagos virškinamumas 57 - 71 %.

Raktažodžiai: ankštiniai, raudonieji dobilai, liucerna, virškinamumas, maistinė vertė.

Introduction. The objective of forage production is the preparation of high-quality, well-preserved animal feed of a high nutritive value. For this purpose, the appropriate forages, as well as the best preparation and conservation technologies, are chosen. In the climatic conditions of Estonia, grass feeds are mostly produced by ensiling. While grasses are normally ensiled relatively well or satisfactorily, ensiling of legumes is more risky. Low ensiling ability of legumes is related to their low sugar content, high buffering ability and high humidity

content (McDonald et al., 1991; Pahlow et al., 2002). To improve the ensiling of legumes, chemical conservants are used (Tamm et al., 1999; Lättemäe, 2000).

Legumes are binding nitrogen from the air, thus sparing the costs on mineral N-fertilizers. In recent years, more legumes have been cultivated to improve and maintain the soil fertility. Estonia has long-term experience of lucerne and clover cultivation. Both diploid and tetraploid cultivars of red clover, and the local varieties of hybrid lucerne suitable for growing in our

climatic conditions have been bred at the Jõgeva Plant Breeding Institute. The yield of tetraploid red clover varieties exceeds that of others due to their higher yield of green mass (Bender, 2000). While the interests of breeders are more focused on yield, the objective of the present investigation has been to study the chemical composition, nutritive value of different red clover and hybrid lucerne varieties, and the possibilities of making high-quality silage.

Legumes are characterized as plants with a high protein and lignin content, and with a relatively low cell wall matter content. Although they contain alkaloids, tannins and estrogens, these factors are considered to be of secondary importance. Van Soest (1994) has reported that moderate tannin content may even improve the protein utilization in the rumen of the ruminants. In the growth period, the digestibility of legumes decreases more slowly than that of grasses, and the optimum time for silage preparation is longer (Kaldmäe and Vadi, 2000). The digestibility of red clover decreased by 0.15% a day, whereas that of rye grass decreased by 0.5% a day (Sheldrick et al., 1995).

The nutritive value of silages is mostly affected by the chemical composition of the material and the digestibility of nutritives, which may be highly variable (from 35% to 80%), depending on different conditions (Lopez et al., 2000). In the present study the effect of different varieties and growth stage of red clover and hybrid lucerne on the chemical composition, digestibility and nutritive value of silage were investigated.

Materials and methods. Samples of legumes were collected from trial fields of the Jõgeva Plant Breeding Institute in 2000 and 2001.

The chemical composition, nutritive value and digestibility of silage prepared from the early-maturing red clover (*Trifolium pratense* L. *subvar. praecox* Witte) varieties 'Varte' (4x) and 'Jõgeva 433' (2x), the late-maturing red clover (*Trifolium pratense* L. *subvar. serotinum* Witte) varieties 'Ilte' (4x) and 'Jõgeva 205'

(2x), hybrid lucerne (*Medicago varia* Mart) varieties 'Karlu' and 'Jõgeva 118' in different phases of development were studied. For this purpose test silages were prepared. Legumes were cut at the height of 5 cm, chopped into 2 cm pieces, supplemented with additive AIV 2000 and conserved into jars. In 90 days the jars were opened; the chemical composition of test silages was determined according to the generally accepted methods, and nutritive value was calculated. The samples of silage and raw materials were analysed in the Institute of Animal Science of the Estonian Agricultural University. The dry matter degradability, as well as organic matter digestibility, of raw material of silages was determined *in vitro* by the method of filter bags using DAISY II and ANKOM analysers and ashes in a mufflekiln.

The results were statistically processed by programme Excel 2000, and on the basis of calculated regression equations, the quality of silage samples analysed in the laboratory was evaluated.

Results and discussion. The early-maturing red clover varieties reached the bud formation stage on June 18 and the late-maturing ones - on July 11, 2001. In 2000, it was nine days. The beginning of flowering of the early red clover varieties was on June 25, and that of late red clover varieties on July 16. As for hybrid lucerne, its bud formation took place on June 25, and early flowering - on July 2. The optimum time for ensiling of the legumes is at the stage of bud formation and at the beginning of flowering (Kaldmäe et al., 2000).

The chemical composition and nutritive value of different varieties of red clover and hybrid lucerne at the direct cut are presented in Table 1.

The crude protein content of the tetraploid red clover variety 'Varte' (197 g/kg) exceeded that of a standard variety 'Jõgeva 433' by 44 g/kg, whereas the crude protein content of 'Ilte' variety was by 20 g/kg lower, compared with a standard variety 'Jõgeva 205'. Due to the warm weather and rainstorms in July, the plants of the late-maturing clover variety 'Ilte' were lodged. While

Table 1. Chemical composition and nutritive value of red clover (RC) and lucerne (L) varieties, bred in Estonia, at the stage of bud formation (BF) and early flowering (EF)

Items	RC "Varte"(4x)		RC "Jõgeva 433"(2x)		RC "Ilte" (4x)		RC "Jõgeva 205" (2x)		L "Karlu"		L "Jõgeva 118"	
	BF	EF	BF	EF	BF	EF	BF	EF	BF	EF	BF	EF
Dry matter, g/kg	122	148	162	171	130	145	160	175	165	199	169	204
In dry matter, g/kg												
crude protein	197	153	169	141	134	148	154	153	201	156	162	142
crude ash	107	93	94	84	112	92	89	80	85	76	93	88
crude fibre	208	256	234	265	271	290	269	282	316	334	375	360
NDF	405	403	410	424	455	438	452	455	432	475	484	481
ADF	284	298	269	318	356	337	344	334	341	382	405	382
N-free extractives	457	470	472	482	442	426	446	439	373	401	350	370
metab. energy, MJ/kg	10.5	10.1	10.6	10.1	10.4	10.3	10.7	10.4	10.5	10.0	10.3	9.9
Dry matter degradability <i>in vitro</i> , g/kg	618	618	626	601	607	581	568	582	586	566	560	538
Organic matter digestibility <i>in vitro</i> , %	81	78	78	76	78	72	74	74	75	71	70	68

lodging their lower leaves dried and fell down. Similar situation was also observed during long-term trials between 1994 and 1996 (Bender, 2000a). It was also confirmed by slightly higher crude fiber content of the 'Ilte' variety.

The dry matter content of tetraploid red clover varieties was extremely low at the stage of bud formation and early flowering – in 'Varte' 122 g/kg and 148 g/kg, and in 'Ilte' 130 g/kg and 145 g/kg, respectively (Table 1). Touri et al. (1996) also reported the dry matter content of 150 g/kg at the stage of bud formation, and 200 g/kg at the stage of early flowering. The remarkably bigger leafage of tetraploid red clover varieties affected the dry matter content. The rainfalls and temperature had also an impact on the dry matter content.

Table 1 shows that the nutritive value and organic matter digestibility of red clover and hybrid lucerne varieties are affected both by the variety and the growth stage. All the red clover and hybrid lucerne varieties contained 0.1 to 0.5 MJ/kg more metabolizable energy at the stage of bud formation, compared with the stage of early flowering. Similarly, their organic matter

digestibility was also better at the bud formation stage – in red clover varieties by up to 5.9% and in hybrid lucerne varieties by up to 4.4%, respectively.

The data on silage made from the tetraploid variety 'Varte' was not used, as due to the low dry matter content the silage was of poor quality.

The low fermentability coefficient (FC) for all direct-cut legumes shows that they would be very difficult to ensile, indicating the need to take measures to prevent butyric acid fermentation, which is unavoidable at a FC around 27. The FC of 36, corresponding to a slightly wilted forage, is still not sufficient to guarantee a good fermentation quality. Only the higher wilt to c. 400 g DM kg⁻¹ resulted in FC values above the critical threshold of 45 (Pahlow et al., 2002).

The dry matter content of the late-maturing red clover variety 'Ilte' was also very low, but due to its lower crude fiber content it was more appropriate for ensiling.

The chemical composition, organic matter digestibility and nutritive value of red clover and hybrid lucerne varieties, related to the stage of herbage, are presented in Table 2.

Table 2. Chemical composition and digestibility of legume silages

Raw material and variety, development stage of silage	Dry matter g/kg	Crude protein g/kg	Crude Fibre g/kg	NDF g/kg	ADF g/kg	ME MJ/kg	OMD* %
Red clover							
Jõgeva 433 (2x)							
bud formation, n=3	168	198	194	331	226	10.0	71
early flowering, n=5	188	164	247	394	279	9.3	64
Jõgeva 205 (2x)							
bud formation, n=3	158	173	274	482	350	10.1	58
early flowering, n=3	161	164	302	479	371	9.5	57
Ilte (4x)							
bud formation, n=3	124	174	278	486	336	10.0	62
early flowering, n=3	142	169	273	457	327	9.4	60
Lucerne							
Karlu							
bud formation, n=4	184	225	275	393	303	10.0	67
early flowering, n=6	193	184	329	472	380	9.3	59
Jõgeva 118							
bud formation, n=3	170	190	331	493	386	9.8	60
early flowering, n=3	186	165	360	516	415	9.1	57

*OMD = in vitro x 0.82 (Kaldmäe et al., 2001)

The trial results revealed that the metabolizable energy content of legume silage, prepared at the stage of bud formation, was from 9.8 to 10.1 MJ/kg, depending on the variety. The metabolizable energy content of legume silage prepared at the stage of early flowering was from 9.1 to 9.5 MJ/kg. Thus, its nutritive value was by 0.6 to 0.7 MJ/kg lower than that of the silage prepared at the stage of bud formation.

Both the nutritive value and organic matter digestibility of silages are significantly affected by the

stage of growth and variety of ensiled legumes, and by the chemical composition, particularly the proportion of fiber fractions. The strongest correlation was found between organic matter digestibility and crude fiber fraction content of legume silages. A strong negative correlation was observed between organic matter digestibility and crude fiber ($r = -0.747$, $P < 0.001$), organic matter digestibility and NDF ($r = -0.891$, $P < 0.001$), and organic matter digestibility and ADF ($r = -0.859$, $P < 0.001$). A positive correlation was revealed between crude protein

and organic matter digestibility ($r = -0.581$, $P > 0.01$). While the organic matter digestibility of the silage prepared from red clover variety 'Jõgeva 433' was 71% at the bud formation stage, and 64% at early flowering, then that of the 'Jõgeva 205' variety was 58% and 57%, and that of 'Ilte' variety was 62% and 60%, respectively. The differences in digestibility appeared also in the hybrid lucerne varieties (Table 2). The organic matter digestibility of red clover was from 59.9 to 63.9% in the UK, 65.0 to 70.1% in Sweden and 71.8 to 72.1% in Finland, respectively. The organic matter digestibility of lucerne in the UK was 63.6% (Dewhurst et al., 2002).

Conclusions. The nutritive value of legume silages depends on the variety and especially on the stage of development of plants. The crude protein content of the tetraploid red clover (*Trifolium pratense* L. subvar. *praecox* Witte) variety 'Varte' was significantly higher (197 g/kg), and the crude fiber content was lower (208 g/kg) at the stage of bud formation, compared with other varieties. Due to the extremely low dry matter content of the tetraploid red clover varieties (from 122 to 130 g/kg at the stage of bud formation and from 145 to 148 g/kg at the stage of early flowering), they should be wilted for ensiling, if possible, or used as green fodder. To achieve the better quality of silage, the legumes should be wilted for ensiling up to the dry matter content of 40%.

The digestibility of legume silage depends on the species, variety and the stage of development. The best digestibility of 71% at the stage of bud formation, and 64% at the stage of early flowering was observed in silage, prepared from the early-maturing red clover (*Trifolium pratense* L. subvar. *praecox* Witte) variety 'Jõgeva 433'.

The hybrid lucerne (*Medicago varia* Mart) variety 'Karlu' exceeded significantly the standard variety 'Jõgeva 118', both in nutritive value and digestibility.

The growing of the late-maturing red clover varieties in Estonia is highly affected by air temperature and rainfalls, and their lower leaves may fall down or even rotten, decreasing their nutritive value.

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