Estimation of red clover (*Trifolium pratense* L.) forage quality parameters depending on the variety, cut and growing year

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ABSTRACT

A field trial was conducted to determine the nutritive value of eight Slovak and Czech red clover varieties and to quantify the effects of variety, cut and year on red clover forage quality. The differences between forage quality of the evaluated red clover varieties were significant for protein supplied when energy is limited in the rumen (PDIE), intestinal digestibility of rumen non-degraded protein (dsi), net energy values and ash content ($P < 0.01$). The achieved results show that the surpassing forage quality was given by diploid Viglana variety with high protein and energy values. The forage of tetraploid varieties Javorina and Dolina was outstanding in protein content, but had the lowest energy values. The nutritive value was significantly affected by cut. The first cut provided forage with a significantly lower ($P < 0.01$) crude protein (CP) content, CP digestibility, degradability of CP (degNL), protein supplied when nitrogen is limited in rumen (PDIN), PDIE and with a significantly higher crude fibre content and energy values ($P < 0.01$). The differences between varieties were more pronounced in the second and in the third cut. Significantly higher ($P < 0.01$) PDIE, PDIN, degNL, net energy values and ash contents were found in the first production year.

Keywords: red clover; protein values; energy values

Red clover is a legume well adapted to poorly drained soils and more tolerant to lower soil pH and fertility than alfalfa. The yield potential of red clover is excellent and some red clover varieties can have higher fodder yields than alfalfa. Red clover is considered a short-lived legume, but new modern varieties are productive for three full seasons. Red clover is also of a very good quality as to its nutritive value and ensiling (Hoffman and Broderick 2001).

Forage quality is affected by many independent factors including maturity, crop species, harvest and storage, environment, soil fertility and variety. Generally, red clover varieties differ in quality according to ploidy level and earliness. A decrease of protein content from early to late populations was reported by Makarenko and Pribytkov (1989). The tetraploid red clover is characterised by a higher content of protein, WSC, potassium and phosphorus and by a lower content of fibre than diploid red clover (Bieniaszewski and Fordoński 1996). As a result of the evaluation of CP, PDIN, PDIE, ME and NEL contents, Fojtík et al. (2001) also found that tetraploid red clover provided higher forage quality as compared to diploid.

The environment also significantly influences the nutritive value of forages. As Buxton (1996) indicated, plant environment modifies the impact of forage maturity resulting in year-to-year, seasonal and geographical effects on forage quality, even when harvested at the same stage of maturity. The documented seasonal variation in forage quality suggests that stability analyses for forage quality include cutting-to-cutting stability within a year as well as year-to-year stability (Sheaffer et al. 1998).

By the establishment of new systems of forage quality evaluation, crude protein fails to characterise the true value of feed nitrogen for dairy cows, because it does not reflect the true protein absorbed in small intestine. The nutritive value of feed proteins may be expressed best by the amount of aminoacids as absorbed in small intestine either from the feed protein which escaped to degradation in rumen and/or from the microbial protein synthesized in rumen using utilisable nitrogen and energy. This is one of the reasons why the French Protein (PDI) system was adopted (Bohane et al. 2003). The PDI system ascribes two protein values to feedstuff, the PDIE value (protein supplied when energy is limited in the rumen) and PDIN value (protein supplied when nitrogen is limited in the rumen). In terms of new systems of energy evaluation in forage, the estimation of metabolizable energy and net energy is also very important.

The near infrared reflectance spectroscopy (NIRS) has become widely recognized as a valuable tool in the accurate determination of the chemical composition of a wide range of forages and in determination of digestibility, protein degradation and energy values (De Boever 1998, Park et al. 1998). Compared with conventional laboratory procedures, NIRS offers advantages of accuracy, speed, simplicity, reduced chemical waste and cost-effective prediction of forage quality components (Cozzolino and Labandera 2002).

The main objectives of presented study was (1) to determine nutritive values of some Slovak and Czech red clover varieties using NIRS method, (2) to compare forage quality of evaluated varieties and (3) to quantify the effects of variety, cutting and production year on red clover forage quality.

MATERIAL AND METHODS

The forage quality of six tetraploid (Javorina, Sigord, Amos, Dolina, Beskyd, Margot) and two diploid (Viglana, Manuela) red clover varieties coming from a red clover trial of the world collection is evaluated in this paper. The experiment was conducted during three years (2000–2002) at the experimental station of the Research Institute of Plant Production in Piešťany. The trial site situated at the altitude of 163 m is located in continental climate and features fluctuating temperatures and relatively unevenly distributed precipitations. Regarding climate the region is warm and slightly dry featuring annual temperature (above 10°C) sums of 3000–2500°C. Its long-term temperature mean is 9.2°C and total annual precipitation 595 mm.

The year 2001 was extraordinarily warm. The average temperature in the course of vegetation period was by 2.0°C higher than the long-term average, with May, July and August exceptionally warm. As regards precipitations year 2001 was normal, with irregular distribution; June was dry and August was extremely dry. Year 2002 was also warmer than average with annual average temperature by 2.6°C higher than the long-term average. The vegetation period was especially warm with July and August being the warmest months. Regarding precipitations year 2002 was normal, the vegetation period featured unevenly distributed precipitations; May and July were the driest months.

The soil at the experimental station site is Haplic Phaeozem, characterised as clay-loamy soil. The evaluation of the soil nutrients using method Mehlich II have shown high magnesium and calcium reserves, medium phosphorus reserves and medium to low potassium reserves. The experimental design was a randomised complete block in three replicates. The plot size was 2.25 m^2. The plots were cut once in the sowing year and three times in the second and third year. The varieties were harvested in comparable growth stage, at the beginning of flowering. The rate of flowering plants of all varieties was recorded at each harvest.

In 2001 and 2002 1-kg samples in two replicates were taken from each cutting. The herbage samples for quality assessment were dried in a drier at a temperature of 60°C and homogenised in laboratory grinder. Ground samples were then analysed at the Research Station of Grassland Ecosystems in Jevíčko, using near infrared reflectance spectroscopy (NIRS). Analysis included crude protein (CP) content, the digestibility of CP, crude fibre (CF), fat and ash. NIRS method was applied also to determine crude protein not degradable in rumen (PDIE – protein supplied when energy is limited in rumen, PDIN – protein supplied when nitrogen is limited in rumen), degradability of crude protein (degNL), intestinal digestibility of rumen non-degraded protein (dsi) and energy values (NEL – net energy of lactation, NEV – net energy of fattening, ME – metabolizable energy).

Two separate sets of analyses were conducted on forage quality data using ANOVA procedure. The first set of analyses was done for individual cuts using two-factor analyses of variance with varieties and years as factors. The three-way ANOVA was performed on all quality parameters, assuming variety, cut and year effects. The Tuckey's test was applied to test the significance of differences between varieties.

RESULTS AND DISCUSSION

The diploid varieties were about 3 to 4 days earlier than the tetraploid ones. The differences between individual varieties presented 1 to 4 days; the order of varieties according to the time of flowering was as follows: Viglana, Manuela, Beskyd, Margot, Sigord, Javorina, Amos, Dolina.

The variety differences in the contents of evaluated quality parameters (Table 1) in the first cut were not significant. The effect of variety was more pronounced in the second cut for ash, degNL, NEL, NEV and ME ($P < 0.01$) and in the third cut for CP, ash, dsi, digestibility of CP, PDIN ($P < 0.05$), PDIE, NEL, NEV and ME ($P < 0.01$). Our results showed no significant variety differences between standard forage quality parameters. A more expressive differentiation between evaluated varieties was found in PDIN, PDIE, dsi, degNL and in energy values, which confirms the need of a complex estimation of nutrients in feeds.

Following the mean values of crude protein content for years and cuts (data not presented) Javorina variety featured the highest CP content and digestibility of CP with the highest values in the third cut. In respect of degradability of crude protein (degNL) the averaged values of the investigated varieties varied from 81.0 to 82.0%. From the nutritional point of view the values are relatively high. The intestinal digestibility of rumen non-degraded protein (dsi) varied between 73.0% (Viglana, Javorina, Dolina varieties) and 75.0% by Manuela variety. These results correspond to the values of intestinal digestibility defined for red clover (73–75%) as indicated by Sommer et al. (1994).

According to the tables of feed nutritional value used to calculate nutrient requirements (Sommer et al. 1994), the optimum PDIN and PDIE values for red clover cut between flower buds formation and beginning of flowering stages are 108–125 g/kg and 89–95 g/kg, respectively. The mean PDIN values determined in our trial varied between 110.47 g/kg (Beskyd variety) and 116.30 g/kg (Dolina variety), those of PDIE varied between 79.40 g/kg (Sigord variety) and 82.48 g/kg (Manuela variety). When compared with the table values, the PDIN results achieved by the tested varieties are at optimal level; those of PDIE are slightly below the established limit. As indicated by Bohane et al. (2003) a better balancing of PDIN and PDIE in the diet should result in a more efficient conversion of dietary N to milk or meat and less excretion of surplus N.

The energy concentration, expressed for dairy cows in MJ NEL/kg DM, is very important. The highest possible level of energy concentration is a prerequisite to feed highly performing cows successfully. But possibilities to increase cows' energy intake by increasing the energy concentration are limited. The energy value of feed in our trial expressed by ME, NEL and NEV values varied from 8.82 to 9.46 MJ/kg (ME), 5.33 to 5.77 MJ/kg (NEL) and from 5.08 to 5.60 MJ/kg of dry matter (NEV). The highest energy values were shown by diploid varieties Manuela and Viglana. The differences between varieties were significant and the variances among maximum (Manuela variety) and minimum NEL and NEV values (Javorina) were 0.44 and 0.54 MJ/kg of dry matter, respectively. The enhancement of NEL value is very important, as Hrabě et al. (2003) reported that an increase of NEL of clover-grass silage by 0.5 MJ/kg DM reduces the need for complementary feed by 2.5 kg per day.

With respect to milk production it is required that feed contain as little of fibre and ash as possible. Manuela diploid variety, containing 234.48 g/kg and 114.01 g/kg DM of fibre and ash, respectively, matches this aspect best. As regards ash contents there were significant differences between varieties, the difference in fibre contents between two extreme values was 6.45 g/kg DM. The mean values of fat content varied between 18.20 and 19.08 g/kg of dry matter.

The results of analyses of variance (Table 2) revealed a statistically significant effect of the cut sequence on forage quality. The cut was an important source of variability for all qualitative parameters apart from fat content. Hakl et al. (2003) explained the variety differences in forage quality of alfalfa by a different maturity stage and different stand development to another cut. The inverse relationship of advancing forage maturity and declining of forage quality is well established. However, Sheaffer et al. (1998) indicated on the basis of their experiment that ranking of alfalfa entries for forage quality was more affected by season than by either location or maturity of the alfalfa. Also Hall et al. (2000) found no connection between growth period or sampling time and forage quality at alfalfa cultivars. The findings of their research indicated that the difference in quality between the high quality and traditional alfalfa cultivars was not due to differences in morphological development.

Crude protein, digestibility of CP, PDIE and PDIN contents in our experiment were the lowest in the

Table 1. Forage quality parameters of red clover varieties in individual cuts averaged across the production years

Variety	CP (g/kg)	Fat (g/kg)	CF (g/kg)	Ash (g/kg)	degNL (%)	dsi (%)	Dig. CP (g/kg)	PDIN (g/kg)	PDIE (g/kg)	ME (MJ/kg)	NEL (MJ/kg)	NEV (MJ/kg)
1st cut												
Viglana	180.68	18.43	245.21	105.92	81.00	75.00	119.14	104.72	80.64	9.67	5.91	5.76
Manuela	178.02	18.63	243.76	105.19	81.00	76.00	114.98	103.57	81.72	9.83	6.03	5.90
Javorina	181.28	18.00	250.72	114.31	80.00	75.00	120.81	103.85	77.57	9.13	5.54	5.33
Sigord	172.85	17.78	251.50	107.39	81.00	75.00	110.77	100.77	78.40	9.47	5.78	5.61
Amos	169.58	18.74	249.68	109.45	81.00	75.00	106.40	99.46	78.98	9.49	5.79	5.63
Dolina	178.78	19.07	247.57	114.29	81.00	75.00	118.72	103.55	78.19	9.28	5.65	5.46
Beskyd	173.64	18.19	248.64	109.10	81.00	75.00	110.19	100.83	78.81	9.45	5.76	5.60
Margot	168.52	18.44	248.01	105.56	81.00	76.00	104.88	99.31	79.33	9.65	5.90	5.76
Mean	175.42	18.41	248.14	108.90	80.88	75.25	113.24	102.01	79.21	9.50	5.80	5.63
HSD (0.05)	38.92	4.78	43.97	19.58	4.09	4.77	41.47	21.97	6.66	0.73	0.51	0.62
HSD (0.01)	53.14	6.53	60.03	26.73	5.59	6.52	56.61	29.99	9.10	0.99	0.69	0.85
2nd cut												
Viglana	192.45	18.33	239.78	113.99	82.00	73.00	139.61	113.70	81.94	9.24	5.62	5.41
Manuela	194.88	18.96	226.98	118.76	83.00	75.00	143.76	117.13	82.74	9.36	5.71	5.53
Javorina	196.65	18.58	228.73	124.93	83.00	74.00	146.50	118.26	81.23	8.98	5.44	5.22
Sigord	191.56	19.18	227.80	128.59	83.00	75.00	141.26	114.56	80.89	8.93	5.41	5.19
Amos	203.50	18.21	221.94	124.60	84.00	74.00	154.37	122.14	82.25	9.10	5.53	5.33
Dolina	207.94	19.23	222.79	128.63	83.00	74.00	160.55	124.24	81.46	8.95	5.42	5.20
Beskyd	189.74	18.71	229.57	125.35	83.00	75.00	139.73	115.06	80.89	8.98	5.45	5.23
Margot	199.31	18.50	223.33	119.69	84.00	74.00	147.25	120.20	83.13	9.33	5.68	5.50
Mean	197.00	18.71	227.62	123.07	83.13	74.25	146.63	118.16	81.82	9.11	5.53	5.33
HSD (0.05)	22.75	4.27	21.90	19.89	3.08	4.38	24.84	15.78	4.65	0.84	0.56	0.67
HSD (0.01)	31.07	5.83	29.89	27.15	4.21	5.98	33.92	21.54	6.34	1.16	0.77	0.92
3rd cut												
Viglana	202.53	20.48	231.35	125.99	82.00	72.00	156.56	126.47	82.01	8.86	5.36	5.11
Manuela	189.62	18.04	232.71	118.07	82.00	74.00	139.47	119.92	82.97	9.19	5.58	5.37
Javorina	204.93	19.48	235.70	133.37	83.00	71.00	161.95	126.40	79.98	8.35	5.01	4.70
Sigord	192.12	18.01	243.50	128.35	82.00	71.00	146.63	118.77	78.92	8.33	4.99	4.67
Amos	195.58	18.66	237.41	126.58	82.00	72.00	150.33	121.92	79.95	8.59	5.18	4.90
Dolina	195.00	18.30	242.21	127.63	82.00	71.00	149.48	121.12	79.49	8.37	5.02	4.71
Beskyd	182.97	17.70	242.05	126.66	82.00	72.00	134.45	115.52	79.44	8.55	5.14	4.86
Margot	201.42	19.03	238.54	130.53	82.00	71.00	156.06	124.19	79.60	8.35	5.01	4.69
Mean	195.52	18.71	237.93	127.15	82.13	71.75	149.37	121.79	80.30	8.57	5.16	4.88
HSD (0.05)	23.64	3.90	13.59	13.92	2.69	3.27	33.60	11.69	3.14	0.80	0.55	0.66
HSD (0.01)	32.27	5.33	18.56	19.00	3.68	4.47	45.87	4.29	4.29	1.10	0.75	0.90

Table 2. Mean squares for red clover nutritive quality

Source of variation	*df*	CP	Fat	CF	Ash	degNL	dsi
Year	1	10775.80**	17.39*	2592.52**	278.63**	11.34**	15.04**
Error A	1	87.02	4.09	34.74	43.57	0.05	0.36
Cut	2	4652.04**	0.97	3368.70**	2935.04**	38.32**	108.04**
Error B	2	59.04	7.50	97.08	66.63	0.96	1.17
Variety	7	209.68	0.95	52.91	157.00**	0.84	2.60*
Year × variety	7	94.05	0.56	140.82	14.61	2.08	2.57*
Cut × variety	14	130.40	1.86	93.08	40.28	0.91	1.65
Year × cut × variety	14	106.31	3.05	83.21	50.22	0.78	1.53
Residual error	47	125.34	1.99	80.68	34.34	1.20	1.13
CVe		5.91	7.58	3.78	4.90	1.34	1.44
Source of variation	*df*	Dig. CP	PDIN	PDIE	NEL	NEV	ME
Year	1	2991.67**	506.97**	23.07**	10.11**	10.12**	0.43**
Error A	1	94.19	13.65	2.24	0.05	0.06	0.09
Cut	2	12949.80**	3549.07**	55.04**	3.27**	4.62**	6.88**
Error B	2	97.63	12.69	0.38	0.01	0.01	0.02
Variety	7	318.92	53.29	14.18**	0.26**	0.35**	0.56**
Year × variety	7	110.42	42.44	4.99*	0.04	0.07	0.10
Cut × variety	14	191.70	40.20	2.07	0.03	0.05	0.07
Year × cut × variety	14	149.73	30.14	2.00	0.03	0.04	0.06
Residual error	47	172.04	34.22	2.40	0.03	0.04	0.07
CVe		9.62	5.13	1.93	3.15	3.79	2.39

Error A – replicate within a year; Error B – replicate within cut within a year; *CVe* (%) – the variation coefficient of experimental error; *, **significant at the 0.05 and 0.01 probability levels, respectively

first cut and were increasing depending on varieties, in the second or the third cut (Figure 1). This confirmed the results of Sheaffer et al. (1998) who also found the highest content of crude protein in the third cut. The highest degNL values were found in the second cut for all varieties. Only in the case of dsi a slightly decreasing trend from the first to the last cut was found.

As for the content of energy in forage in the course of vegetation period the knowledge reported by Belyea et al. (1999) about the effects of cuttings on the energy value of forages was confirmed. In all varieties the energy values gradually decreased from the first to the third cut (Figure 2). Similarly, Hakl et al. (2003) found significantly higher net energy contents at the first cut in comparison to remaining cuts. The content of CF was the highest at the first cut, which has also been confirmed by the results by Wiersma et al. (1998) about low content of crude protein and high content of fibre in fodder cut in spring. Griffin et al. (1994) supposed that this is owing to a slower reduction of leaf/stem ratio in summer as compared with spring.

The variety × cut interactions for forage quality were not significant, reflecting that variety ranking was relatively consistent across cuttings. Hall et al. (2000) reported that the non-significant interaction between the alfalfa cultivars and growth periods indicates that the higher forage quality of high-quality cultivars is a stable phenotypic trait throughout the growing season.

Several authors (Buxton 1996, Wiersma et al. 1998, Tamm and Bender 2003) described the influence of growing year and environmental conditions on the quality of red clover forage. The effect of growing year on the nutritive value of

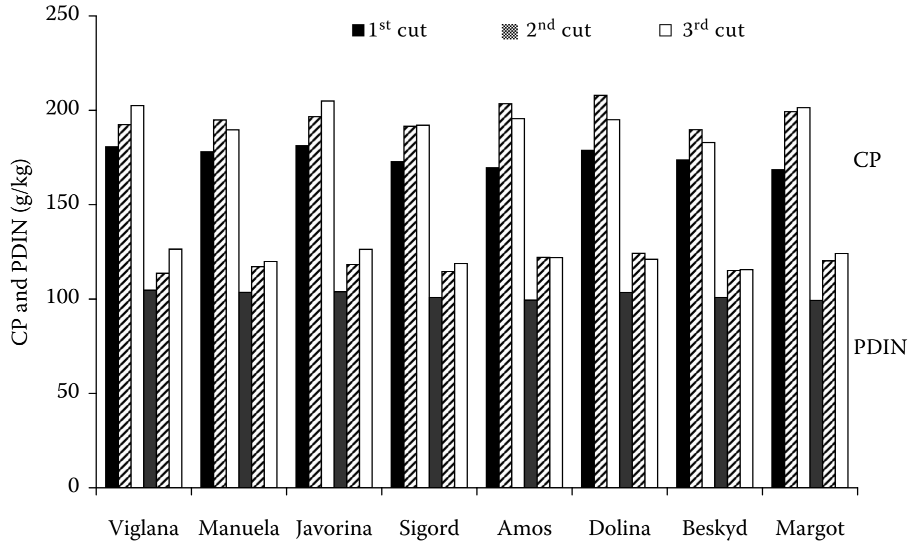

Figure 1. The changes of protein values (CP, PDIN) in individual cuts

investigated varieties was confirmed in our trial, as well. The analyses of variance revealed that all quality parameters were significantly influenced by year, while higher contents of PDIE, PDIN, degNL, NEL, NEV and ash were found in the first production year (Table 3). The order of the examined varieties according to the determined parameters was different depending on the production year, which indicates that varieties respond to weather conditions differently.

The achieved results have shown that the good forage quality was provided by diploid Viglana variety, featuring high protein values and at the same high-energy values of the feed. Tetraploid Javorina and Dolina varieties had higher contents of CP and digestibility of CP, but the lowest energy values. The forage of Manuela variety provided lower protein values, but the highest energy values per kilogram of dry matter. The forage quality of Amos and Margot varieties were equal. The varieties Sigord and Beskyd provided forage with low quality parameters. These findings indicate that forage quality of evaluated varieties did not correspond exactly to the growth stage at harvest. Fojtík et al. (2001), who investigated the effect of sequential harvesting of red clover on forage qual-

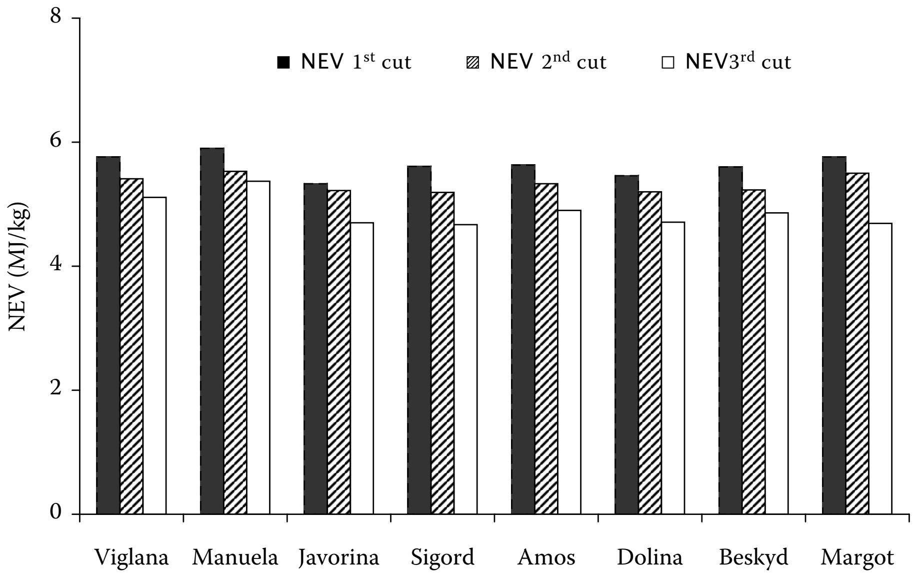

Figure 2. The net energy of fattening (NEV) content in individual cuts

Table 3. Nutritive value of red clover varieties in the first and the second production year

Variety	CP (g/kg)		Fat (g/kg)		CF (g/kg)		Ash (g/kg)		degNL (%)		dsi (%)	
	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
Viglana	178.70	205.07	18.56	19.59	230.33	247.22	119.01	111.58	82.00	80.00	73.00	73.00
Manuela	176.87	198.13	17.96	19.12	232.84	236.12	115.94	112.07	82.00	81.00	73.00	76.00
Javorina	184.64	203.93	17.97	19.40	234.48	242.28	125.10	123.30	82.00	81.00	73.00	73.00
Sigord	171.92	199.09	17.83	18.81	236.19	245.67	123.90	118.98	82.00	82.00	73.00	74.00
Amos	178.83	200.28	18.05	19.01	231.87	240.81	121.47	118.94	82.00	82.00	73.00	74.00
Dolina	187.13	200.68	18.77	18.95	228.04	247.00	125.77	121.25	82.00	81.00	73.00	73.00
Beskyd	168.60	195.62	17.78	18.61	239.94	240.23	120.92	119.81	81.00	82.00	73.00	75.00
Margot	183.04	196.45	18.53	18.78	227.87	245.39	119.12	118.06	83.00	81.00	73.00	74.00
Mean	178.72	199.91**	18.18	19.03*	232.70	243.09**	121.40**	118.00	82.00**	81.25	73.00	74.00**
HSD (0.05)	4.46		0.65		3.70		2.48		0.44		0.43	
HSD (0.01	5.95		0.87		4.94		3.31		0.59		0.58	
Variety	Dig. CP (g/kg)		PDIN (g/kg)		PDIE (g/kg)		NEL (MJ/kg)		NEV (MJ/kg)		ME (MJ/kg)	
	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
Viglana	129.15	147.71	115.04	114.88	81.97	81.09	5.93	5.32	5.73	5.12	9.43	9.07
Manuela	128.12	137.35	114.99	112.08	82.23	82.72	5.99	5.54	5.80	5.40	9.52	9.39
Javorina	139.33	146.84	118.63	113.70	79.91	79.27	5.66	5.00	5.41	4.75	9.04	8.60
Sigord	123.49	142.27	112.40	110.33	79.52	79.27	5.71	5.07	5.47	4.84	9.10	8.70
Amos	131.31	142.75	117.32	111.69	81.05	79.72	5.84	5.15	5.63	4.94	9.30	8.82
Dolina	140.01	145.81	121.63	110.97	81.24	78.19	5.74	4.98	5.52	4.73	9.16	8.56
Beskyd	120.28	135.97	111.09	109.85	79.52	79.91	5.72	5.17	5.49	4.97	9.13	8.85
Margot	134.91	137.21	119.14	109.98	81.97	79.40	5.94	5.11	5.75	4.88	9.45	8.76
Mean	130.83	141.99**	116.28**	111.69	80.93**	79.95	5.82**	5.17	5.60**	4.95	9.27**	8.84
HSD (0.05)	5.24		2.32		0.61		0.07		0.08		0.10	
HSD (0.01)	6.99		3.10		0.82		0.09		0.11		0.14	

*. **significant at the 0.05 and 0.01 probability levels, respectively

ity, found that tetraploid red clover is extremely flexible crop and can be harvested at a whole range of maturity stages.

On the basis of the knowledge about different nutritive values of diploid and tetraploid red clover varieties we compared the assessed varieties also in this respect. From our results we may deduce that diploid varieties (Viglana, Manuela) can also produce high quality fodder. This is confirmed by the statement of Míka et al. (1997) who indicated that breeding of diploid red clover varieties with higher forage quality parameters than tetraploid varieties is possible. The highest net energy per kilogram of dry matter contents was found at both diploid varieties and when compared with some tetraploid varieties, the higher content of protein values and less fibre were observed.

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