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YIELDS AND QUALITY OF GREEN FORAGE FROM RED CLOVER DI- AND TETRAPLOID FORMS

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Abstract

The purpose of the study has been to determine the yield, chemical composition and nutritive value of green forage from di- and tetraploid forms of red clover grown in different seed density regimes. The yield of green forage was analysed in the first year of full use, in 2003 and 2004, having cut the plants in the early inflorescence phase (1st cut). In green forage samples, the following were determined: basic chemical composition, concentration of water soluble carbohydrates (WSC), fibre fractions (NDF, ADF and ADL) and macronutrients (P, K, Mg, Ca, Na). The nutritive value of green forage was expressed according to the INRA 1988 system.

The yield of green matter from the tetraploid cultivars Bona and Jubilatka was higher than from the diploid cultivars Krynia and Parada. The cultivar Bona distinguished itself by its highest average yield of green matter, dry matter, total protein and net energy. The dry matter content was the most variable green forage quality characteristic. In both years, green forage from the diploid cultivars contained more dry matter than the one made from the tetraploid varieties. At the same time, seed density did not have any significant influence on the differentiation of green forage yields.

Green forage from the two diploid cultivars was characterised by a similar energy and protein value and a better fill unit value (better potential intake by ruminants). The concentrations of phorphorus, potassium and calcium in green forage from the diploid forms were slightly higher compared to the tetraploid forms, unlike the level of magnesium, which was slightly lower. Irrespectively of the polidy level, the concentration of macronu-

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trients, except phosphorus, was higher in the first year of the study. Considering the nutritional requirements of animals, green forage from the analysed red clover cultivars was characterised by an inadequate level of magnesium, deficient amount of sodium and an improper Ca:P ratio. The results suggest that diploid forms of red clover can potentially generate a higher nutrititive value than tetraploid ones.

Key words: red clover, forage quality.

PLONY I JAKOŚĆ ZIELONKI DI- I TETRAPLOIDALNYCH FORM KONICZYNY CZERWONEJ

Abstrakt

Celem badań było określenie plonowania oraz składu chemicznego i wartości pokarmowej zielonki di- i tetraploidalnych odmian koniczyny czerwonej uprawianej z zastosowaniem różnej gęstości siewu nasion. Plon zielonki analizowano w pierwszym roku pełnego użytkowania w latach 2003 i 2004, po skoszeniu roślin w fazie początku kwitnienia (I pokos). W próbach zielonki oznaczono podstawowy skład chemiczny, zawartość cukrów rozpuszczalnych w wodzie (WSC), frakcje włókna (NDF, ADF i ADL) i makroelementy (P, K, Mg, Ca, Na). Wartość pokarmową zielonki wyrażono według systemu INRA 1988.

Plon zielonej masy odmian tetraploidalnych Bona i Jubilatka był wyższy niż odmian diploidalnych Krynia i Parada. Wyróżniała się odmian Bona, dla której stwierdzono najwyższy średni plon zielonej masy, suchej masy, białka ogólnego i energii netto. Cechą jakościową zielonki o największej zmienności była zawartość suchej masy. W obu latach użytkowania zielonka odmian diploidalnych miała wyższą zawartość suchej masy niż zielonka odmian tetraploidalnych. Jednocześnie gęstość siewu nasion nie miała istotnego wpływu na zróżnicowanie plonów zielonki.

W przypadku zielonki odmian diploidalnych stwierdzono zbliżoną wartość energetyczną i białkową oraz korzystniejszą wartość wypełnieniową (większa możliwością pobrania przez przeżuwacze). Poziom fosforu, potasu i wapnia w zielonkach odmian diploidalnych był nieznacznie wyższy, a magnezu niższy w porównaniu z odmianami tetraploidalnymi. Niezależnie od poziomu ploidalności odmian koncentracja makroelementów, z wyjątkiem fosforu, była wyższa w pierwszym roku badań. Uwzględniając wymagania pokarmowe zwierząt, zielonka badanych odmian koniczyny miała odpowiedni poziom magnezu, niedoborową ilość sodu oraz niewłaściwy stosunek Ca:P. Uzyskane wyniki wskazują, że odmiany diploidalne koniczyny czerwonej mają możliwości osiągania wyższej wartości pokarmowej niż odmiany tetraploidalne.

Słowa kluczowe: koniczyna czerwona, jakość zielonki.

INTRODUCTION

Next to grasses, red clover is a leading forage crop grown in the moderate climate zone (Lee et al. 2004). The advantages of cultivating red clover are such as its high fodder value, mixed cultivation with grasses, barley, oat or wheat, its positive role in crop rotation systems by binding nitrogen, restricting the development of cereal diseases ("disease braek") and providing natural protection from monocot weeds. Moreover, red clover has bene-

ficial influence on the natural environment as it prevents erosion, acts as a phytomelioration plant and enhances the landcsape's aesthetic value (Bawolski, Ścibor 1982, Duer 1999, Hogh-Jansen, Schjoerring 1997, Kostuch 1998, Spancer, Todd 2003, Steiner et al. 1995).

The main storage components accumulated by red clover are total protein, which can make up as much as 21% of the dry matter, and fibre (between 18-26% of d.m.). Both di- and tetraploid forms of red clover are grown commercially. The two forms have different morphological traits and agricultural characteristics. In a study conducted by Tomaszewski Junior (1989), the yield of grain from diploid forms was 30-60% higher than from tetraploid ones. Likewise, Jaranowski and Broda (1986) as well as Bruździak et al. (1989) concluded that diploid cultivars are more productive. However, tetraploid cultivars can possess superior qualitative composition. Żuk-Gołaszewska et al. (1999), who analysed yields of di- and tetraploid cultiars of red clover, demonstrated that red clover tetraploid forms were superior in production of total protein yields. At present, the quality-orientated breeding of clover focuses on improving the content and quality of proteins (the amino acid composition, sensitivity to proteolysis, PPO activity), delaying the ligification process as plants mature or increasing the share of carotens and decreasing the concentration of anti-nutritional compounds, e.g. isoflavones (Lee et al. 2004).

The objective of this study has been to determine the level of yields and composition of green forage, including the feed value, obtained from red clover di- and tetraploid cultivars.

MATERIAL AND METHODS

The research was based on plant material such as green forage made from di- and tetraploid cultivars of red clover, grown in strict field experiments conducted in 2003 and 2004 on experimental fields at the Experimental Station in Bałcyny. Two-factor experiments with different forms of red clover (diploid cultivars Krynia and Parada, tetraploid cultivars Bona and Jubilatka) sown in different seed density regimes (4, 8, 12, 16 kg ha^{-1}) were set up in a split-plot design. The yield of green forgae was determined in the first year of full use, during the early inflorescence phase (1^{st} cut). Samples of green forage weighing 0.5 kg were taken from ten places on each plot and then an average analytical sample was extracted. Next, the samples were dried up for 24 h at 60°C in a forced air-flow dryer (manufactured by BINDER) and comminuted in a high-speed grinding mill (made by FOSS). The content of dry matter, crude ash, total nitrogen, ether extract, crude fibre was determined according to the AOAC procedures (2005). The concentration of water soluble carbohydrates (WSC) was determined with the an-

throne method (THOMAS 1977) whereas NDF, ADF and ADL fibre fractions were assayed using GOERING and VAN SOEST'S method (1970) in an ANKOM 220 apparatus. The content of true protein was determined using trichloroacetic acid (LICITRA et al. 1996). For determination of the content of ash components, comminuted material was mineralised in concentrated sulphur acid with addition of chlorine dioxide as an oxidising agent. The concentration of phosphorus was determined by colorimetry using the vanadium-molybdenum method. Potassium, calcium and sodium were determined with the photoflame method (ESA) and the concentration of magnesium was assayed using the absorption atomic spectrophotometry (AAS).

The feed value of green forage was derived from the chemical composition, according to the INRA's fodder evaluation system (WINWAR programme, KOWALSKI, KAŃSKI 1993) and expressed in the following units: UFL (unit of energy for milk production), PDIE (protein digested in the small intestine depending on rumen fermented organic matter), PDIN (protein digested in the small intestine depending on rumen degraded protein) and FUC (fulfilment unit for cows).

The results of the green forage yield volumes and chemical analyses underwent statistical analysis using the correlation and regression methods as well as analysis of variance. For comparison of the treatment means, T Tukey's test was applied and HSDs (honest significant differences) were established. The evaluation of groups of means was performed using orthogonal contrasts (ELAND 1964). Statistcal hypotheses were verified at the level of significance $p < 0.05$.

RESULTS AND DISCUSSION

The first year of the experiment was warm and dry. The average of the temperatures during the plant growing season was higher than the mean long-term temperature and the precipitaion in the spring months (MARCH, April) was lower or close to the multi-year average. The weather conditions in 2004 were more favourable to the vegetative development of red clover. After a wet winter, profuse rainfall appeared in April, becoming more and more intensive in all the consecuitive months of the growing season. In May and June, the rainfall was 87.1 and 90.6 mm, respectively, thus being higher than the long-term average (56.7 and 68.3 mm) ŻUK-GOŁASZEWSKA et al. 2006a.

The yield of red clover green matter was significantly different between the cultivars in each year; likewise, the year x cultivar interaction was significant (Table 1). The weather conditions, being more favourable to the vegetative growth of plants in 2004, raised the green forage yield by an average 12.3 t ha^{-1} compared to 2003.

Table 1

Green forage yield of di- and tetraploid red clover cultivars grown in different sowing density regimes in the two years of the experiment (t h^{-1})

Cultivars (C)	Sowing density (S) (kg ha^{-1})	Year (Y)		Mean
		2003	2004	OxG
Krynia	4	38.5	52.3	45.4
	8	42.7	51.4	47.0
	12	40.1	49.8	44.9
	16	43.5	51.0	47.2
Parada	4	43.6	53.7	48.6
	8	44.1	45.6	44.8
	12	44.6	44.8	44.7
	16	44.3	47.1	45.7
Bona	4	48.7	61.2	54.9
	8	45.9	63.2	54.5
	12	49.2	61.0	55.1
	16	46.7	63.9	55.3
Jubilatka	4	42.3	55.4	48.8
	8	43.4	62.9	53.2
	12	32.6	61.8	47.2
	16	42.6	64.6	53.6
		YxC		O
Krynia		41.2	51.1	46.1
Parada		44.1	47.8	46.0
Bona		47.6	62.3	55.0
Jubilatka		40.2	61.2	50.7
		YxS		G
	4	43.2	55.6	49.4
	8	44.0	55.8	49.9
	12	41.6	54.4	48.0
	16	44.3	56.7	50.5
		L		
		43.3	55.6	

$HSD_{0.05}$ for: Years (Y) – 5.79; Cultivars (C) – 3.77, YxC – 6.42; Sowing density(S) – n.s.; LxG – n.s.; OxG – n.s.; LxOxG – n.s.

In general, the tertraploid cultivars Bona and Jubilatka yielded higher than the diploid cultivars Krynia and Parada. The highest average yield of green matter, found for cv. Bona, was significantly higher than generated by all the other varieties. However, the response in yield of green matter produced by the analysed red clover cultivars depended on the weather conditions in the two years of the tests (Figure 1). In 2003, the cultivar Bona produced a higher yield than all the other cultivars but in 2004 yielded like the tetraploid cultivar Jubilatka. Stable albeit the lowest yields were produced by the diploid cultivar Parada. In the present study, differences in the

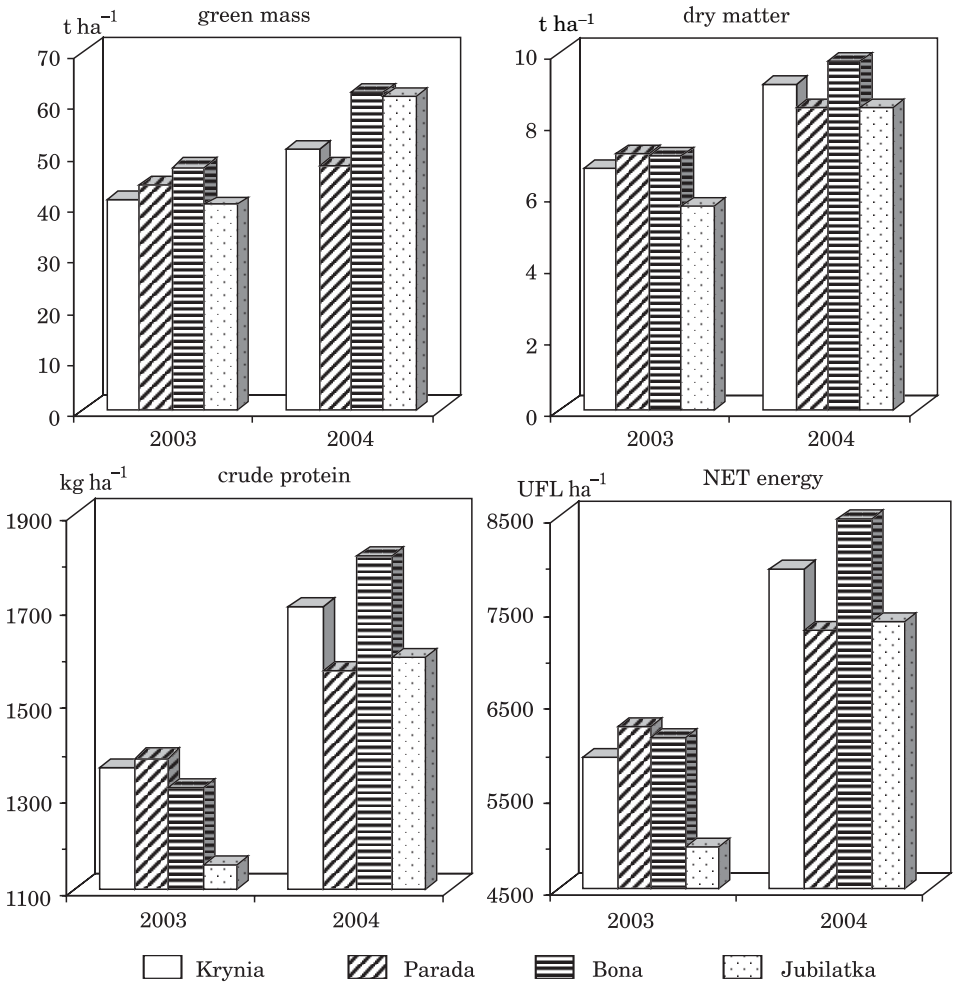

Fig. 1. Green mass, dry matter, crude protein and net energy in di- and tetraploid red clover cultivars

yields of green matter found between the years may have been caused by the difference in the rainfall during the growing season of red clover. Analogously to the study reported by LETO et al. (2004), differences in yields produced by di- and tetraploid cultivars did not exceed 10%. In turn, GRAMAN (1988) attained a 10-60% higher green matter yield from tetraploid cultivars than from diploid ones in years characterised by very high precipitation. The correlation between green and dry matter in diploid cultivars in both years of the trials was significant, which is confirmed by results of some previous studies conducted by MUNTEAN and SAVATTI (2003).

The net energy yields were correlated with yields of dry matter, which was associated with approximately the same energy value of dry matter in all the cultivars. The smallest differences in protein yields may have been caused by a higher content of protein in the cultivars producing lower yields (Figure 1).

The chemical composition of the analysed green forage produced from red clover was differentiated, although statistically proven differences appeared oly for the content of dry matter, neutral detergent fibre (NDF) and acid detergent lignin (ADL) – Tables 2 and 3.

Among the analysed traits, dry matter was most variable. The concentration of this yield component was found to differ between the years of harvest, ploidy status and between the two diploid cultivars Krynia and Parada. In both years, more dry matter was found in green forage from the diploid cultivars than from the tetraploid ones. At the same time, the tetraploid cultivars responded differently to the weather conditions prevailing during the experiment. The cultivar Bona, similarly to the diploid cultivars, generated less dry matter in green forage in 2003. whereas cv. Jubilatka, which generally contained low amounts of dry matter, produced more of this component in 2004 (significant comparison *years x tetraploid cultivars*).

Significant differences in the content of structural sugar fractions appeared in the groups of di- and tetraploid cultivars. In both years of the trials, the NDF fraction in the green forage from the diploid cultivar Parada was higher than in the green forage from cv. Krynia; by analogy, the tetraploid cv. Jubilatka contained more NDF than cv. Bona. The higher level of NDF in the two cultivars mentioned above may be a result of their higher content of polysaccharides (hemicelluloses), which ensure high digestibility of fodder by ruminants (PURWIN 2007). The ADL fraction in green forage, which indicates the lignification degree, was different in the two tetraploid cultivars. Likewise, the diploid cultivars were characterised by a different response in terms of the content of the ADL fraction in the years of the experiment.

The differences in the content of total protein and true protein were relatively small (within the error limit), but noteworthy was the differentiation in the concentration of protein in the diploid cultivars depending on the course of the weather conditions during the tests.

Table 2

Means for the chemical composition of di- and tetraploid red clover cultivars in the years of the experiment (g kg^{-1} d.m.)

Cultivars	Year	Dry matter	Ash	Organic matter	Crude protein	True protein	True protein/ /crude protein	Ether extract	WSC	NDF	ADF	ADL
	2003	165	111	888.6	200	136	70.0	23.8	94.1	407	337	45.6
Krynia	2004	178	111	889.0	187	139	74.7	21.9	103.7	454	321	41.0
	mean	172	111	888.8	193	138	72.3	22.8	98.9	430	329	43.3
	2003	163	111	889.2	193	146	76.0	18.9	76.6	445	333	31.0
Parada	2004	177	113	886.8	185	139	75.2	21.0	99.8	500	336	45.5
	mean	170	112	888.9	189	143	75.6	19.9	88.2	472	334	38.2
	2003	150	114	885.7	185	119	64.6	21.1	78.1	392	325	31.7
Bona	2004	156	112	888.0	186	140	74.9	21.8	94.9	454	325	38.4
	mean	153	113	886.8	185	130	69.7	21.4	86.5	423	325	35.0
	2003	142	111	888.6	202	144	71.4	21.2	108.8	489	299	42.8
Jubilatka	2004	138	111	888.8	188	141	75.2	20.3	99.0	499	332	52.9
	mean	140	111	888.7	195	143	73.3	20.7	103.9	494	316	47.8
SEM		5.71	5.62	6.26	9.32	12.52	4.97	4.21	16.65	31.74	14.61	5.38

Table 3

Estimates of contrasts between treatments in analyses of the chemical composition of green mass

Contrasts	Dry matter	Ash	Organic matter	Crude protein	True protein	True protein/ /crude protein	Ether extract	WSC	NDF	ADF	ADL
Between years	-6.2*	0.7	-0.5	-3.8	-3.7	-0.3	12.6	-2.5	3.6	4.4	-1.3
Diploid cvs vs. tetraploid cvs	-49.7*	2.2	-2.0	-0.5	-2.9	-1.5	11.0	-2.2	10.7	-13.7	-2.0
Diploid cvs: Krynia vs Parada	1.0	-2.4	2.4	-1.5	-4.4	-1.6	1.9	5.4	-39.8*	-7.4	3.1
Tetraploid cvs: Jubilatka vs Bona	-11.1*	-0.8	1.1	4.4	2.8	-0.2	15.4	1.1	44.7*	5.1	9.8*
Years vs ploidy	21.1*	6.4	-5.9	-5.1	-10.6	-3.4	9.8	15.0	24.0	-27.2	-13.7
Years vs diploid cvs	-1.3	-0.4	0.4	-6.0	-8.5	-2.2	17.4	3.0	13.1	14.8	15.0*
Years vs tetraploid cvs	13.8*	-0.1	0.6	4.7	2.0	-1.0	-11.0	-5.9	-0.4	-3.6	-9.7

* significant at $p < 0.05$

PURWIN (2007) reports that the concentration of water soluble carbohydrates (WSC) is a factor that determines the suitability of green forage for ensiling and their enery value. It is assumed that tertraploid cultivars are better regarding the nutritive value compared to diploid forms. In general, they contain more protein and water soluble carbohydrates but less fibre (BIENIASZEWSKI FORDOŃSKI 1996). In the present study, the results are ambiguous. However, we noticed a certain tendency towards a higher concentration of water soluble carbohydrates in the second year of the tests (2004), which in general was more favourable to the growth of the plants (Table 3).

The feed value of green forage is verified by the energy and protein value, relations between the concentration of crude fibre and water soluble carbohydrates as well as the fulfilment value. The results presented in Table 4 demonstrate that the tested green forage has a similar energy value (UFL) irrespectively of the cultivar and year. The protein value of green forage was more evidently differentiated between the years than between the cultivars, assuming lower values in 2004. A higher value of PDIN compared to PDIE in green forage from all the analysed cultivars is typical of green forage made from papilionaceous plants, which contain relatively little crude fibre and a high level of soluble sugars.

Table 4

Nutritive value of di- and tetraploid red clover cultivars according to INRA (1988)

Cultivars	Year	UFL kg^{-1} d.m.	PDIN (g kg^{-1} d.m.)	PDIE (g kg^{-1} d.m.)	FUC kg^{-1} d.m.
Krynia	2003	0.87	125.0	98.67	0.85
	2004	0.87	117.2	96.42	0.78
	mean	0.87	121.1	97.54	0.82
Parada	2003	0.87	120.9	97.88	0.86
	2004	0.86	115.3	95.17	0.78
	mean	0.86	118.1	96.52	0.82
Bona	2003	0.86	116.0	95.86	0.93
	2004	0.87	116.9	96.69	0.90
	mean	0.86	116.5	96.28	0.92
Jubilatka	2003	0.87	127.1	99.58	0.99
	2004	0.87	118.2	96.88	1.01
	mean	0.87	122.6	98.23	1.00

Noteworthy are big differences between the di- and tetraploid forms in the fulfilment value of the green forage, a parameter which characterises possible intake of bulk feeds by ruminants. A much higher value of fulfilment units (FUC) in green forage from tetraploid forms of red clover is

generally an undesirable parameter as it implies potentially lower capacity for the intake during direct feeding of animals (INRA 1988). A lower fulfilment value of green forage from diploid cultivars is associated with their lower content of dry matter. Similar energy and protein values alongside a lower fulfilment value of diploid cultivars indicate that it is possible to produce good quality fodder from diploid cultivars of red clover. Our results correspond to the conclusions drawn by DROBNA and JANČOVIČ (2006), who suggested that it should be possible to introduce diploid forms of red clover to commercial production owing to their nutritive value being higher than that of tetraploid cultivars.

The weather conditions during the growth of plants and the form of red clover differentiated the concentration of mineral components in green forage (Table 5).

Table 5

Content of macronutrients in di- and tetraploid red clover cultivars (g kg^{-1} d.m.)

Cultivars	Year	P	K	Mg	Ca	Na
Krynia	2003	3.41	38.36	3.54	14.67	0.60
	2004	4.46	37.64	3.15	14.79	0.46
	mean	3.93	38.00	3.35	14.73	0.53
Parada	2003	3.64	43.04	3.37	15.15	0.43
	2004	3.82	39.60	3.14	15.08	0.41
	mean	3.73	41.32	3.26	15.11	0.42
Bona	2003	3.61	40.56	3.85	14.05	0.46
	2004	3.67	38.66	3.23	13.79	0.40
	mean	3.64	39.61	3.54	13.92	0.43
	2003	3.45	39.92	3.80	16.12	0.82
	2004	3.71	39.18	3.07	13.29	0.43
	mean	3.58	39.55	3.43	14.70	0.62

The concentration of phosphorus in green forage in the first year of the tests ranged between 3.41 and 3.64 g in 1 kg d.m., with more phosphorus found in the green forage from cv. Parada (2n) and Bona (4n). In the second year, the concentration of phosphorus was higher, and the effect exerted by the form of red clover became more evident. The biggest difference (over 30%) between the two years of the tests was found for the diploid cultivar Krynia, whereas the smallest one (1.7%) appeared for the tetraploid cv. Bona. In general, the diploid forms were characterised by a slightly higher concentration of phorphorus than the tetraploid ones. Contrary relations in the concentration of phorphorus in di- and tetraploid cultivars were determined by BIENIASZEWSKI and FORDOŃSKI (1996), who found out that tetraploid forms

contained more phosphorus and potassium than diploid ones. In our experimet, the content of potassium was shaped differently. In both years, cv. Parada had the highest concentration of potassium and, irrespectively of the cultivar, the concentration of potassium was higher in 2003. The concentrations of magnesium were shaped analogously, but the tetraploid cultivars contained more of this element. In these cultivars, in the second year of the tests, a large difference as found, namely 18.9% for cv. Bona and 23.5% for cv. Jubilatka. This, however, does not undermine the finding that the content of magnesium in all the types of green forage analysed, regardless the type of red clover or the year of the experiment, fully covered the nutritional demands of animals, especially dairy cows, which – according to the INRA – demand 1.5 to 2.0 g Mg kg^{-1} d.m. of fodder. When analysing the concentration of calcium in the two consecuitive years of the experiment, it was demonstrated that the tetraploid forms (cv. Bona and Jubilatka) were characterised by a higher level of this nutrient in 2003 (1.8 and 21.3% more, respectively) than in 2004. No effect of the year on the level of calcium in the diploid cultivars was discovered. Generally, the average concentration of calcium was the highest in green forage from cv. Parada (15.11 g kg^{-1} d.m.) and the lowest one – in cv. Bona (13.9 g kg^{-1} d.m.).

Considering the nutritional usability of forage, the concentration of calcium in red clover was twice as high as required by animals, which confirms the suggestion expressed by PŁAZA et al. (1009) that cultivation of red clover in a pure sowing regime does not provide full quality fodder in terms of the content of mineral components. It is so because the quality of fodder is determined by mutual ratios between particular mineral elements (Figure 2). In the present study, the Ca:P ratio in both years of the tests was widened and ranged within 2.57-3.62:1, while the optimum ratio is 1.5-2.0:1 (the INRA). At the same time, it was demonstrated that cv. Jubilatka in the first year of the experiment and cv. Krynia in both years were characterised by the most favourable K:(Ca+Mg). As SABA et al. (2000) claim, high content

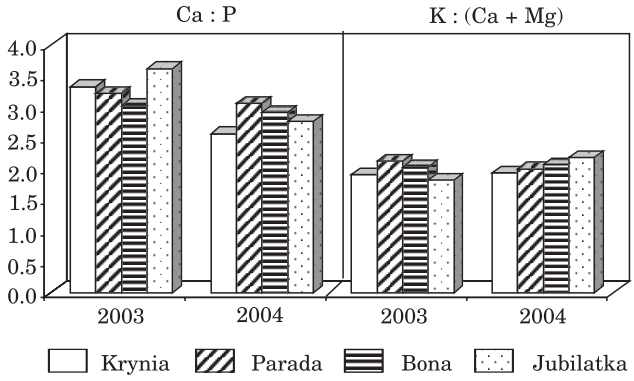

Fig. 2. The ratios of Ca:P and K:(Ca + Mg) in di- and tetraploid red clover cultivars

of potassium, due to the antagonistic metabolic effect of this element on magnesium, may consititue an additional factor which depresses the bioavailability of magnesium. Sodium deficit acts in a similar fashion. In the analysed types of green forage (Table 5), the level of sodium was deficient compared to the nutritional demand of animals, becase the optimum values according to the INRA are 1.7-2.0 g kg^{-1} d.m. Higher concentrations of this element were found in the green forage made in the first year of the experiment. The average content of sodium was the lowest in cv. Parada (2n) and Bona (4n).

CONCLUSIONS

1. Yields of green forage from the first cut of red clover of the tetraploid cultivars are 15% higher than produced by the diploid varieties. The highest average yields of green matter, dry matter, total proten and net energy were obtained from cv. Bona red clover.

2. In the green forage from the diploid cultivars, the concentration of phosphorus, potassium and calcium was higher in the tetraploid cultivars, unlike the content of magnsium, which was lower. Irrespecyively of the ploidy level, the concentration of macronutrients, except phosphorus, was lower in the year which favoured the vegetative development of plants.

3. The above results suggest that diploid forms of red clover can potentially achieve a higher nutritive value than tetraploid cultivars.

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