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EFFECTS OF A CULTIVAR, HARVEST TIME AND NITROGEN DOSE ON THE CONTENT OF NITROGEN, PHOSPHORUS AND POTASSIUM IN THE BIOMASS OF *LOLIUM PERENNE* L.*

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ABSTRACT

The study was conducted in 2012–2015. A small-area field experiment was performed at the Agricultural Experiment Station in Tomaszkowo (53°42'40.8"N 20°26'04.7"E) owned by the University of Warmia and Mazury in Olsztyn. The aim of this experiment was to determine the effects of a cultivar, nitrogen dose and harvest time on the content of nitrogen (N), phosphorus (P) and potassium (K) in the biomass of perennial ryegrass. The content of N, P and K in biomass was determined as follows: N – by the Kjeldahl method, P and K – by flame photometry. During the three-year study, the diploid cultivar Bajka accumulated higher amounts of N, whereas the tetraploid cultivar Baronka accumulated significantly higher amounts of K. The tested perennial ryegrass cultivars did not differ in P content, determined on a dry matter basis. It was found that the N content of plants was higher when perennial ryegrass was harvested at noon and in the afternoon. The reverse was observed with regard to K accumulation. Perennial ryegrass biomass harvested in the morning was characterised by significantly higher K content than the biomass harvested at noon and in the afternoon. Harvest time had no influence on P concentrations in the analysed cultivars. The accumulation of N and K in perennial ryegrass was affected by N fertilisation. In both cultivars, the N and K content of plants was significantly higher in treatments fertilised with N, whereas no differences were found between N doses. A minor increase in the P content of the analysed cultivars occurred only when the N dose was doubled.

Keywords: perennial ryegrass, diploid, tetraploid, harvest time, nitrogen fertilisation, macronutrients.

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INTRODUCTION

Lolium perenne L. Is one of the most valuable fodder grasses. It is widely cultivated in many regions of the temperate zone. Perennial ryegrass has high feed value and is well adapted to temperate climates, which is why it is the main forage grass species grown in Europe (SMITH et al. 2005, LEE et al. 2007). In Poland, it is commonly used as cut fodder, and numerous perennial ryegrass varieties have been entered into the National List of Agricultural Plant Varieties.

The mineral content of ruminants' diets affects the health status, body condition and productivity of animals (PIRHOFER-WALZL et al. 2011). Roughage, the main component of a feed ration, is a major source of minerals (DACCORD et al. 2001). However, it is difficult to formulate balanced diets with the desired chemical composition because the concentrations of minerals in plants are determined by various factors, such as the species composition of sward (STANIAK, KSIEŻAK 2008, WYLUPEK et al. 2014), climatic conditions (HU 2007, BEDNAREK et al. 2009, SILVA et al. 2011), season of the year, harvest time (PIRHOFER-WALZL et al. 2011, SCHLEGEL et al. 2016) and fertilisation (DACCORD et al. 2001, SODER, STOUT 2003, HØGH-JENSENET et al. 2006, BUMANE 2010). An insufficient supply of minerals may lead to physiological dysfunction in animals. Nitrogen (N) is the main component of protein, and high-yielding animals have high protein requirements. Phosphorus (P) plays an important role in the development of bones, the skeletal system and the nervous system, and it significantly influences the fertility and milk performance of cattle. Potassium (K) present in intracellular fluids regulates osmotic pressure, the acid-base balance, carbohydrate and protein metabolism as well as the water balance in tissues. It also activates nerve cells, supports proper muscle function and controls the electrical activity of the heart. Feeding mineral-rich green fodder to animals eliminates the need for additional mineral supplements, thus reducing production costs (SCHLEGEL et al. 2016).

The mineral composition of grassland herbage is determined by many factors, and N fertilisation and cultivar selection play a very important role. Nitrogen participates in plant metabolism and nutrient transport, thus influencing plant growth and development. Research shows that late-maturing varieties, characterised by prolonged vegetative phase relative to generative phase, contain more nutrients. The doubling of chromosome number leads to an increase in cell and vacuole size, and in nutrient accumulation, which improves digestibility and feed value (HUME et al. 2010, BARYŁA, KULIK 2013). The quality of grass biomass is also affected by harvest time (PURWIN et al. 2014). Previous studies investigating the chemical composition of grasses have focused mostly on the effects of fertilisation (KESSLER, JOLIDON 1998, WYSS, KESSLER 2002, BUMANE 2010), harvest date (DACCORD et al. 2001, SUN et al. 2010, SCHLEGEL et al. 2016) and genetic forms (SUGIYAMA 2006,

CONAGHAN et al. 2008). However, the influence of harvest time on the chemical composition of perennial ryegrass remains insufficiently researched (PURWIN et al. 2014).

The objective of this study was to determine the effects of a cultivar, N dose and harvest time on the content of N, P and K in perennial ryegrass dry matter.

MATERIALS AND METHODS

The study was conducted in 2012-2015. A small-area field experiment was performed at the Agricultural Experiment Station in Tomaszkowo (53°42'40.8"N 20°26'04.7"E) owned by the University of Warmia and Mazury in Olsztyn. The experiment had a randomised block design with four replications. It was established on 29 August 2012, on proper brown soil (medium-heavy loam) of quality class IVa and good rye complex (5) according to the Polish soil classification system (Systematyka gleb Polski 1989). Soil pH was neutral (pH_{KCl} 6.6). The soil was characterised by a moderate content of available P (50.6 mg P kg⁻¹ soil) and K (120.4 mg K kg⁻¹ soil), and low magnesium content (46.0 mg Mg kg⁻¹ soil). The soil was moderately abundant in micronutrients: 148.0 mg Mn kg⁻¹, 3.0 mg Cu kg⁻¹, 7.8 mg Zn kg⁻¹ and 1400.0 mg Fe kg⁻¹. The experimental factors were: (i) perennial ryegrass (*Lolium perenne* L.) cultivar (Bajka 2n, Baronka 4n), (ii) N dose (0 – control treatment, 120 kg N ha⁻¹, 240 kg N ha⁻¹), and (iii) harvest time (8.00-10.00, 12.00-14.00, 16.00-18.00). Seeding amounts were 27 kg ha⁻¹ (cv. Bajka) and 38 kg ha⁻¹ (cv. Baronka). The difference in seeding density resulted from ploidy levels and seed mass. Bajka is a late-maturing cultivar that enters the heading stage at the beginning of June, whereas Baronka is a medium-early cultivar, entering the heading stage 7-8 days before Bajka. Before the experiment, P and K fertilisers were applied at 35 kg P ha⁻¹ and 50 kg K ha⁻¹, respectively. In the years of full utilisation, the treatments were fertilised with 35 kg P ha⁻¹ and 100 kg K ha⁻¹. Phosphorus was applied as a single dose in early spring, and K fertiliser was split into two equal doses and applied in spring and after the 1st harvest. Nitrogen fertiliser was applied before the spring growing season and after the 1st, 2nd and 3rd harvest. First-cut herbage was harvested at the beginning of heading of the early cultivar Baronka, and then cutting was carried out every 5 weeks (2nd harvest), 6 weeks (3rd harvest) and 6-7 weeks (4th harvest). The plot size was 10 m². One-kilogram biomass samples were collected after each harvest to analyse the chemical composition of plants. The content of N, P and K in biomass was determined: N – by the Kjeldahl method, P and K – by flame photometry.

The results were processed statistically by repeated measures ANOVA.

The significance of differences between treatment means was determined by the Tukey's test at $p < 0.05$. All calculations were performed using Statistica 12.0 software.

Weather conditions

Rainfall deficiency was noted during sowing and plant emergence (last days of August and in September of 2012) when precipitation levels reached 45.1 mm and 45.7 mm, i.e. 76% and 80% of the long-term average (Table 1). Air temperature approximated the long-term average in August, and slightly

Table 1

Mean air temperature and rainfalls in the years 2012-2015

Years	Mean air temperature (°C)							Mean of the growing season (Apr-Oct)
	Apr	May	June	July	Aug	Sept	Oct	
2012	7.8	13.4	15.0	19.0	17.7	13.5	7.4	13.4
2013	5.9	14.8	17.5	18.0	17.4	11.3	8.9	13.4
2014	8.8	13.0	14.4	20.4	17.1	13.6	8.7	13.7
2015	6.7	11.8	15.5	17.5	19.8	13.5	6.1	13.0
Multi-year	7.7	13.5	16.1	18.7	17.9	12.8	8.0	13.5
Years	rainfalls (mm)							sum of the growing season (Apr-Oct)
	Apr	May	June	July	Aug	Sept	Oct	
2012	73.1	51.7	103.2	121.0	45.1	45.7	68.5	508.3
2013	28.5	54.5	61.2	121.9	37.6	101.1	16.0	420.8
2014	26.0	32.7	50.8	37.3	86.1	25.9	15.1	273.9
2015	38.2	29.7	29.5	81.9	14.3	63.8	19.4	276.8
Multi-year	33.3	58.5	80.4	74.2	59.4	56.9	42.6	405.3

exceeded the long-term average in September. Suboptimal weather conditions during sowing and in the early stages of plant growth had no adverse effect on plant density due to heavy rainfall in the months preceding the sowing. In June and July, precipitation accounted for 128% and 163% of the long-term average, respectively. In the growing season of 2013, mean air temperature approximated the long-term average, and total precipitation was 15.5 mm higher than the long-term average. However, the distribution of rainfall was uneven: a considerable water deficit was noted in June, August and October, whereas total precipitation exceeded the long-term average by 64% and 78% in July and September, respectively. In the growing season of 2014, weather conditions were not conducive to plant growth. Rainfall slightly exceeded the long-term average only in August, whereas considerable rainfall deficiency was observed in the remaining months. Over that period, total precipitation was around 32% lower than the long-term average,

and air temperatures approximated the long-term average. The hydrothermal conditions during the growing season of 2015 were similar. Mean monthly temperatures were close to the long-term average, whereas total monthly precipitation varied widely. A water deficit was noted in May and June, when total rainfall accounted for 51% and 37% of the long-term average. The hydrothermal conditions were optimal in July and September, whereas considerable rainfall deficiency was observed in August and October.

RESULTS AND DISCUSSION

Nitrogen content

The N content of grasses should be around 20 g kg⁻¹ on a dry matter basis (FALKOWSKI et al. 2000). In the tested cultivars of perennial ryegrass, it was determined at 22-23 g kg⁻¹. An analysis of the mean values over three years revealed that the N content was higher (by approx. 3%) in the diploid cultivar Bajka (Table 2). Nitrogen accumulation in plants was affected by N fertilisation. The lower and higher N dose increased the N content of perennial ryegrass by 4% and 28%, respectively. Harvest time also influenced N concentrations, which were lower in plants harvested in the morning (8.00-10.00), and significantly higher in biomass harvested at noon

Table 2

Content of nitrogen, phosphorus and potassium in *Lolium perenne* L. biomass (g kg⁻¹ d.m.)

Treatment	N	P	K
Cultivar			
Baronka	22.2a*	3.6a	23.2b
Bajka	22.9b	3.6a	21.3a
Nitrogen kg ha ⁻¹			
0	20.4a	3.5a	21.3a
120	21.2b	3.5a	22.6b
240	26.1c	3.7b	22.7b
Harvest time			
8 ⁰⁰ -10 ⁰⁰	22.3a	3.6a	22.6b
12 ⁰⁰ -14 ⁰⁰	22.6ab	3.5a	21.9a
16 ⁰⁰ -18 ⁰⁰	22.9b	3.5a	22.1a
Cuts			
I	18.8a	3.3a	23.3b
II	21.6b	3.5b	23.0b
III	23.3c	3.7c	21.4a
IV	26.6d	3.8c	21.1a

* Values within a column marked with different letters are significantly different at $p < 0.05$.

(12.00-14.00) and in the afternoon (16.00-18.00). Nitrogen accumulation in perennial ryegrass increased significantly between the 1st harvest and subsequent harvests, by approximately 15%, 24% and 41%. The above results are consistent with those reported by PIRHOFFER-WALZL et al. (2011). The differences in N concentrations between the 1st harvest and subsequent harvests of grasses may be related to the passage from the generative growth to the vegetative growth of plants (SCHLEGEL et al. 2016).

The responses of perennial ryegrass cultivars to different doses of N fertiliser were similar. The lower N dose (120 kg ha⁻¹) had no significant effect on N accumulation in biomass in cv. Baronka or cv. Bajka, whereas an increase in the fertiliser dose to 240 kg N ha⁻¹ significantly contributed to N accumulation, particularly in cv. Bajka (Figure 1). In comparison with the control treatment, the N content of biomass was 32% higher in cv. Bajka and 23.5% higher in cv. Baronka.

The N content of the analysed cultivars was affected by harvest time.

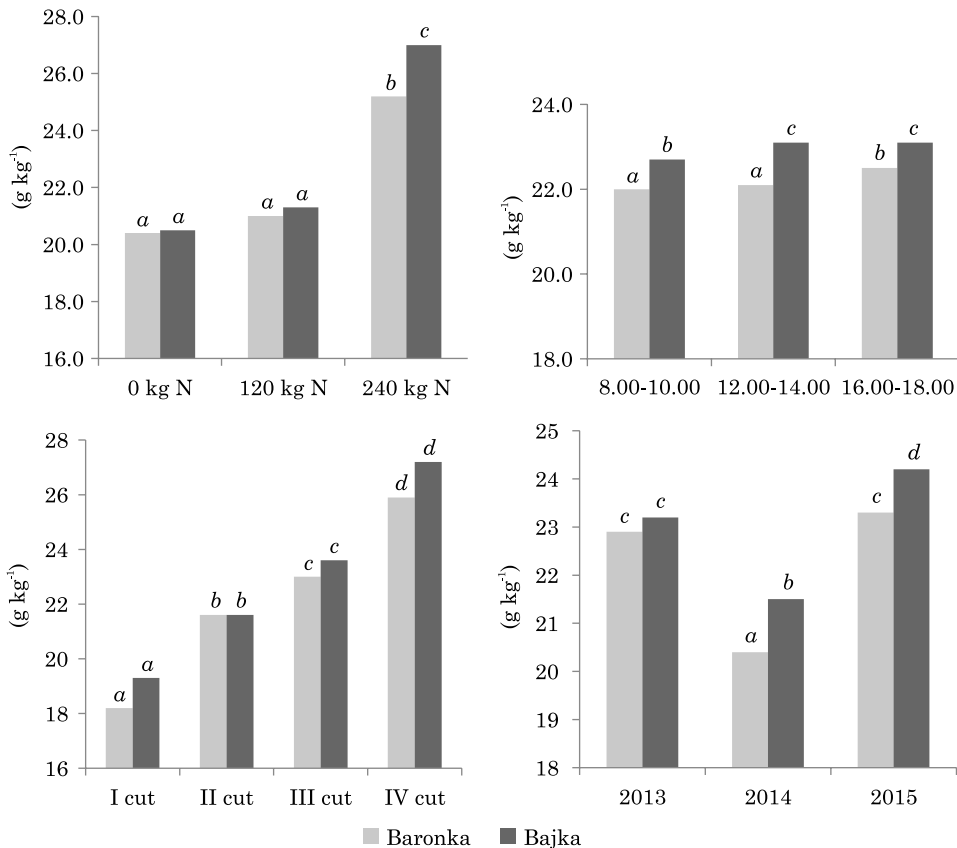


Fig. 1. Effects of nitrogen dose, harvest time and year of the study on the nitrogen content of perennial ryegrass. Means with the same letter were not significantly different ($P < 0.05$)

In cv. Bajka, the N content was higher in the dry matter of plants harvested at noon (12.00-14.00) and in the afternoon (16.00-18.00), but the differences noted between the above results were not statistically significant. In cv. Baronka, the N content of biomass was considerably higher when plants were harvested in the afternoon (16.00-18.00), whereas no significant differences in the N content were found between plants harvested in the morning (8.00-10.00) and at noon (12.00-14.00). The present findings indicate that N accumulation in biomass can be increased by harvesting grasses at noon and in the afternoon, although this is a varietal trait. The N content of the tested cultivars of perennial ryegrass increased significantly between the 1st and subsequent harvests, and it was highest in the 4th harvest in both the diploid and the tetraploid cultivar.

An analysis of the N content of perennial ryegrass cultivars across the years of the study revealed that it was determined by weather conditions. The lowest N content of biomass was noted in the 2nd year of the study, which was not conducive to plant growth and development. Considerable rainfall deficiency during the growing season limited N mobility and accumulation in plants (SILVA et al. 2011, GRZEBISZ et al. 2013). The tetraploid cultivar Baronka was more sensitive to water deficit, and it was characterised by the lowest N content in 2014. Greater water stress susceptibility of tetraploid perennial ryegrass cultivars, compared with diploid cultivars, was also reported by OLSZEWSKA (2006), SUGIYAMA (2006) and TOZER et al. (2017).

Phosphorus content

An analysis of the mean values over three years demonstrated that perennial ryegrass cultivars did not differ in P content, which was determined at 3.6 g kg⁻¹ on a dry matter basis in both Bajka and Baronka (Table 2). According to FALKOWSKI et al. (2000), the above amount is sufficient to meet the P requirements of animals. No significant differences in the P content of biomass were found at a fertiliser dose of 120 kg N ha⁻¹. Only a 100% increase in the N dose contributed to a minor increase in the P content of the tested cultivars. Similar results were reported by WYSS and KESSLER (2002), and TAUBE et al. (1995). In a study by KESSLER and JOLIDON (1998), increasing doses of N fertilisation led to a minor decrease in the P content of meadow grass. In the present experiment, harvest time had no influence on P concentrations in plants. However, the P content of perennial ryegrass varied significantly between the 1st harvest when it was lowest, and the 3rd and 4th harvests when it was highest. An increase in the P content of grass biomass between the 1st harvest and subsequent harvests was also observed by WYSS and KESSLER (2002), and PIRHOFER-WALZL et al. (2011).

The values of the following interactions between the experimental factors: cultivar x fertilisation, cultivar x harvest time, cultivar x harvest, cultivar x year of the study were similar in both cultivars of perennial ryegrass (Figure 2). An analysis of the P content of perennial ryegrass cultivars across

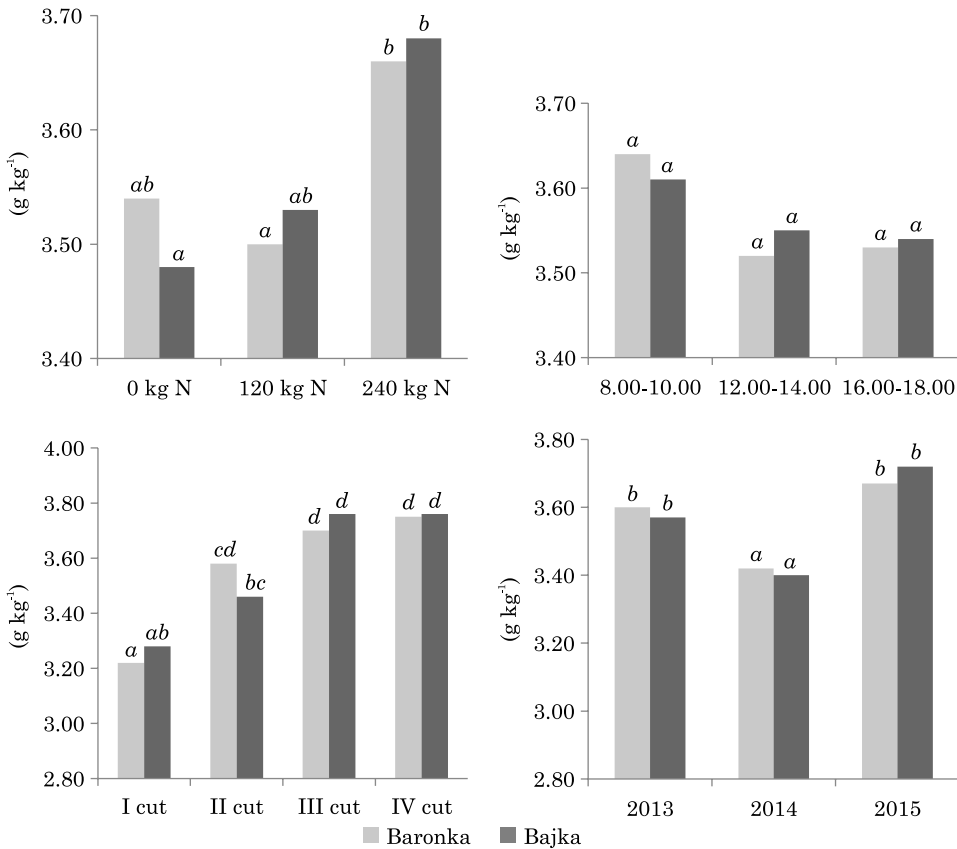


Fig. 2. Effects of phosphorus dose, harvest time and year of the study on the nitrogen content of perennial ryegrass. Means with the same letter were not significantly different ($P < 0.05$)

the years of the study revealed that it was lowest in the 2nd year, whereas no significant differences were found between the 1st and the 3rd year.

Potassium content

During the three-year study, cv. Baronka accumulated significantly higher amounts of K than cv. Bajka (by approximately 9%, Table 2). Perennial ryegrass fertilised with N was characterised by considerably higher K content on a dry matter basis, compared with the unfertilised treatment, whereas no significant differences were noted between N fertiliser doses of 120 kg ha⁻¹ and 240 kg ha⁻¹. In a study by KESSLER and JOLIDON (1998), N fertilisation had no or only a minor effect on the K content of meadow grass. According to STANIAK and KSIEŻAK (2008), K accumulation in plants is affected by the form of N fertiliser. Fertilisation with nitrate N, compared with ammonium N, increases the K content of plants. Perennial ryegrass biomass harvested in the morning (8.00-10.00) had significantly higher K content than biomass

harvested at noon (12.00-14.00) and in the afternoon (16.00-18.00). An analysis of the mean values over three years demonstrated that first-cut and second-cut herbage accumulated more K than third-cut and fourth-cut herbage. The above differences resulted from the fact that K uptake by plants is highest during the period of intensive growth (GRZEBISZ et al. 2013), i.e. during the first and second regrowth of sward.

In the current study, K uptake by perennial ryegrass was affected by N fertilisation (Figure 3). Both cultivars accumulated significantly higher

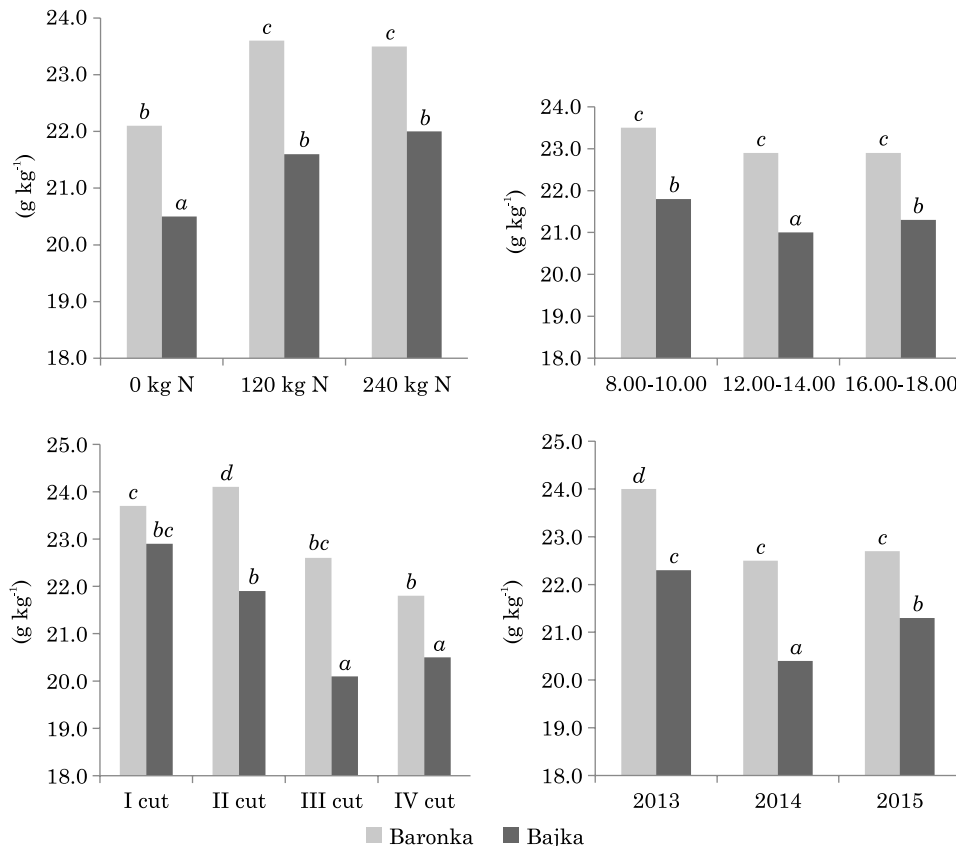


Fig. 3. Effects of potassium dose, harvest time and year of the study on the nitrogen content of perennial ryegrass. Means with the same letter were not significantly different ($P < 0.05$)

amounts of K in N-fertilised treatments, but no differences were found between N doses. In comparison with the control treatment, cv. Baronka fertilised with N had higher K content, by approx. 7% (at the lower N dose) and by approx. 6% (at the higher N dose); in cv. Bajka fertilised with N, K content was higher by approx. 5% and 7%, respectively. In the tetraploid cultivar, harvest time had no significant effect on the K content of perennial ryegrass dry matter, whereas the diploid cultivar had considerably lower K

content when biomass was harvested at noon (12.00-14.00). The K content of the aerial parts of perennial ryegrass varied across harvests. In both cv. Baronka and cv. Bajka, first-cut and second-cut herbage accumulated significantly higher quantities of K than third-cut and fourth-cut herbage. A decrease in the K content was greater in cv. Bajka than in cv. Baronka. Throughout the study, the K content of biomass was highest in the first year of full utilisation, and substantially lower in the 2nd and 3rd year of the experiment, in both cultivars. The decrease in K concentrations resulted from unfavourable hydrothermal conditions during the growing season. Soil moisture deficit reduces K uptake from the soil solution (GRZEBISZ et al. 2013). As a result, nutrient availability decreases and nutrient transport in plants is largely limited (HU et al. 2007, SILVA et al. 2011).

CONCLUSIONS

During the three-year study, the diploid cultivar Bajka accumulated higher amounts of N, whereas the tetraploid cultivar Baronka accumulated significantly higher amounts of K. The tested cultivars of perennial ryegrass did not differ in P content, determined on a dry matter basis. It was found that the N content of plants was higher when perennial ryegrass was harvested at noon and in the afternoon than in the morning. The reverse was observed with regard to K accumulation. Perennial ryegrass biomass harvested in the morning was characterised by significantly higher K content than the biomass harvested at noon and in the afternoon. Harvest time had no influence on P concentrations in the analysed cultivars. The accumulation of N and K in perennial ryegrass was affected by N fertilisation. In both cultivars, the N and K content of plants was significantly higher in treatments fertilised with N, whereas no differences were found between N doses. It was only a 100% increase in the N dose that contributed to a minor increase in the P content of the analysed cultivars. The results of this study indicate that the chemical composition of perennial ryegrass is affected not only by an N dose or a cultivar, but also by the harvest time.

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