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EFFECT OF A HARVEST TIME AND CULTIVAR ON THE CHEMICAL COMPOSITION AND *IN VITRO* RUMINAL DRY MATTER DEGRADABILITY OF PERENNIAL RYEGRASS (*LOLIUM PERENNE* L.)*

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Abstract

The aim of this study was to determine the effect of a harvest time on the chemical composition and dry matter degradability of perennial ryegrass (*Lolium perenne* L.) depending on a swath and cultivar (Bajka – diploid; Baronka – tetraploid). The herbage was harvested at four times of the day (6:00, 10:00, 14:00, 18:00) – the first swath on 15th May 2012, and next after 4, 5 and 6 weeks. Chemical composition and dry matter degradability after 48 hours of *in vitro* incubation were determined. All the analyzed treatments had an impact on the content of DM, crude protein and soluble and structural fractions of carbohydrates. The diploid cultivar had a greater content of DM, WSC and aNDF ($P < 0.01$) and a lesser content of crude protein and ADF ($P < 0.01$) compared with the tetraploid cultivar. Dry matter of the first swath herbage was characterized by the greatest content of WSC and the least content of aNDF ($P < 0.01$). In each consecutive swath, the WSC content significantly decreased ($P < 0.01$) while aNDF increased ($P < 0.01$). The increase in DM content between 14:00 and 18:00 was due to the deposition of products of photosynthesis, which is confirmed by a parallel increase in the concentration of WSC. The effect of treatments on the degradability of DM *in vitro* has not been confirmed, but the results indicate a smaller impact of a cultivar than the harvest management. Numerous interactions between the analyzed treatments indicate the need to choose the harvest time depending on a genotype and swath. Ruminal degradability indicates that high value ryegrass can be obtained regardless of the harvest time during the day. However, greater suitability for ensiling was shown by the diploid cultivar harvested at 10:00 and the tetraploid harvested at 18:00.

Keywords: perennial ryegrass, harvest time, cultivar, *in vitro* dry matter degradability.

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INTRODUCTION

The main issue in nutrition of high yielding cows is how to achieve high energy concentration in feed rations while maintaining an appropriate level of physically effective fiber. Increasing the concentration of energy through an increase in the contribution of roughage or replacing grass silage with maize silage can cause deterioration of the physical structure of feed rations and, consequently, the risk of subclinical acidosis in whole herds, as well as increased feed costs resulting from a higher consumption of protein supplements. Production of grass silage similar in energy value to maize silage can help avoid these risks.

Perennial ryegrass (*Lolium perenne* L.) is the grass with a high energy value and the greatest suitability for ensiling. The energy value results from a high content of water soluble carbohydrates, hemicellulose and a low degree of lignification. The effect of this chemical composition is high rumen degradability of dry matter and feed intake (Miller et al. 2001). A drawback of perennial ryegrass is its ability to accumulate nitrogenous compounds, including ammonia nitrogen, but there is little accumulation of nitrate nitrogen in new cultivars, which are characterized by a greater nitrogen metabolism. Another negative feature of ryegrass is its high sensitivity to frost, which damages the root system, resulting in the reduction of yield and nutritive value of forage (Baryła 2005, Baryła, Kulik 2006).

The breeding work which has been carried out up to this day intends to increase yielding and the potential by introducing more tetraploid cultivars with higher yielding and agriculture value in relation to diploid cultivars (Krzywiecki, Kozłowski 2003), characterized by faster regeneration after winter, higher resistance to diseases, and above all, higher energy value and greater palatability (Czembor 2007, Baryła, Kulik 2013).

The main factor influencing the chemical composition of ryegrass is the stage of vegetative development, which, through changes in carbohydrate fractions, determines the digestibility of organic matter (Bodarski, Krzywiecki 2001). The current laboratory *in vitro* techniques, which are alternatives to *in vivo* studies, ensure relatively accurate estimation of the digestibility of forage implemented at lower cost studies (Holden 1999, Mabjeesh et al. 2000, Wilman, Adesogan 2000).

The research on the chemical composition of herbages concerned mainly the impact of fertilization (Bumane 2010), genetic form (Conaghan et al. 2008) and harvest time (Sun et al. 2010). There is little information on the effect of time of the day on the chemical composition of herbage obtained from perennial ryegrass. The same is true about the interaction between genetic forms of ryegrass and the time of the day.

The experiment was designed to determine the effect of a harvest season on the chemical composition and effective degradability of dry matter of perennial ryegrass depending on a genotype and swath.

MATERIAL AND METHODS

Two perennial ryegrass (*Lolium perenne* L.) cultivars: Bajka (diploid) and Baronka (tetraploid), were grown in Bartążek (53°42′ N, 20°29′ E). Each cultivar was sown in four plots (2 m x 5 m) in the soil complex of wheat defective class IVa, fertilized in the autumn with 30 kg N ha^{-1}, 75 kg of P_2O_5 ha^{-1}, 75 kg K_2O ha^{-1} and with 60 kg N ha^{-1} supplied before the onset of plant growth.

The herbage was first harvested on 15th May (first swath), and next after 4, 5 and 6 weeks at four different times of the day: 6:00, 10:00, 14:00 and 18:00. Immediately after cutting grass from each location, always from the same part of each field, average samples of herbage were collected (1.5 kg). The samples were stored at -25°C and before the analysis they were dried at 40°C using Binder FED 115 drying ovens with forced convection, and ground in the Retsch SK 100 cross beater mill to a particle size of 1 mm.

In vitro ruminal degradability of dry matter was determined using a Daisy II incubator (ANKOM Technology Corporation, Fairport, NY, USA) and F57 polyester filter bags 5.0 cm x 5.5 cm in dimension and with a pore size of about 25 µm (ANKOM). Samples of herbages (0.25 g) were heat sealed in bags and placed in jars. Then, they were incubated in a mixture of buffering solution and rumen fluid prepared in accordance with the recommendations of ANKOM, for 48 hours at 39 ± 0.5°C. In order to obtain anaerobic conditions, every jar was supplied with carbon dioxide for 30 seconds before closing. After the incubation, the bags were washed in cold tap water and rinsed in distilled water in three cycles. Finally, the bags containing incubation residue were analyzed to determine aNDF (Mertens 2002). In order to calculate ruminal degradability of dry matter, *in vitro* procedures recommended by the manufacturer were used (ANKOM Technology Corporation, Fairport, NY, USA).

Samples of herbages were assayed for proximate chemical composition by standard methods (AOAC 2005), while the WSC was assessed by the anthrone method (Thomas 1977). The content of aNDF was determined by the method of Meretns (2002) and ADF and ADL were analyzed by the method proposed by Van Soest et al. (1991) using an Ankom 220. Non-protein nitrogen (NPN) was calculated as a difference between total nitrogen and protein nitrogen determined with the use of trichloroacetic acid (TCA), as described by Licitria et al. (1996).

All data were subjected to three-way Anova, using the GLM procedure of the Statistical Analysis System (SAS 1995). The model describing chemical composition and dry matter degradability of perennial ryegrass herbage accounted for the effects of genotype, swath, harvest time and interactions

$$y = G + P + H + G \times P + P \times H + G \times H + G \times P \times H$$

where:

G – the effect of a genotype; P – the effect of a swath; H – the effect of the time of harvest; $G \times P$ – interaction between the genotype and the swath; $P \times H$ – interaction between the swath and the time of harvest; $G \times H$ – interaction between the genotype and the time of harvest; $G \times P \times H$ – interaction between the genotype, swath and the time of harvest.

The significance of differences between means (genotype, swath, harvest time) was estimated with the Duncan's test.

RESULTS AND DISCUSSION

The influence of a cultivar, swath and harvest time on the chemical composition of perennial ryegrass was shown in Table 1, and the chemical com-

Table 1

Chemical composition and dry matter degradability of perennial ryegrass herbage (% DM)

Treatment	DM (g kg^{-1})	CP	NPN (% TN)	WSC	aNDF	ADF	DMD (%)
Genotype (*G*)							
Diploid	205^{A}	14.4^{B}	42.1	21.3^{A}	52.5^{A}	28.0^{B}	79.0
Tetraploid	173^{B}	14.6^{A}	45.1	20.1^{B}	50.7^{B}	28.6^{A}	80.4
SEM	5.48	0.34	2.19	0.85	0.54	0.35	0.63
Swath (*P*)							
I	173^{A}	17.0^{A}	53.0^{B}	25.8^{A}	48.6^{A}	25.6^{A}	84.7
II	181^{A}	13.2^{B}	46.9^{b}	23.5^{B}	49.5^{B}	28.9^{B}	80.6
III	182^{A}	12.5^{C}	37.5^{Aa}	18.8^{C}	52.2^{C}	28.7^{B}	78.4
IV	219^{B}	15.3^{D}	36.9^{Aa}	14.8^{D}	56.2^{D}	29.9^{C}	75.1
SEM	7.25	0.30	2.64	0.84	0.57	0.35	0.55
Harvest time							
6:00	163^{A}	14.8^{B}	47.6	16.7^{A}	53.2^{A}	29.4^{A}	79.3
10:00	202^{C}	14.2^{A}	40.3	22.2^{BD}	51.9^{B}	27.5^{B}	79.3
14:00	182^{B}	14.8^{B}	45.5	20.5^{C}	50.9^{Ca}	28.1^{C}	80.0
18:00	210^{C}	14.1^{A}	40.8	23.5^{D}	50.5^{Cb}	28.2^{C}	80.2
SEM	6.96	0.50	3.12	1.07	0.77	0.44	0.90
Interactions							
$G \times P$	ns	**	ns	**	**	**	ns
$G \times H$	*	**	ns	**	**	**	ns
$P \times H$	**	**	ns	**	**	**	ns
$G \times P \times H$	ns	**	ns	**	**	**	ns

CP – crude protein, NPN – non-protein nitrogen, WSC – water soluble carbohydrates, DMD – dry matter degradability after 48 hours of incubation *in vitro*; SEM – standard error of the mean; ns – not significant

position of herbage harvested at 6:00, 10:00, 14:00 and 18:00 in the individual harvests of diplo- and tetraploid herbage is shown in Figures 1-6. A significant effect of all the analyzed treatments on the content of DM, crude

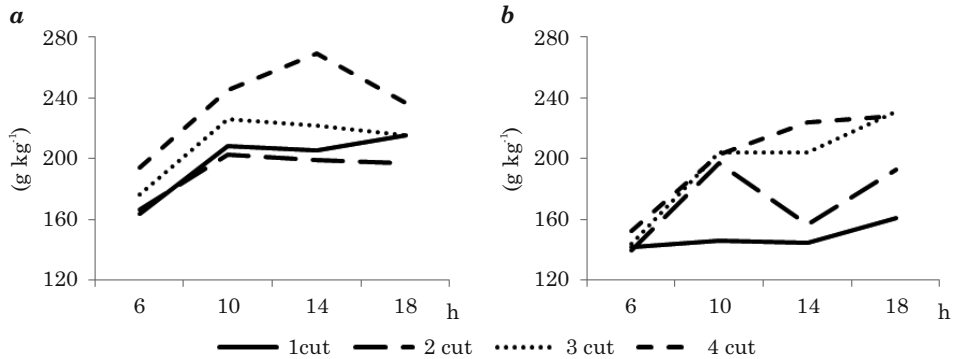

Fig. 1. Dry matter content in diploid (*a*) and tetraploid (*b*) perennial ryegrass herbage harvested at different times od the day

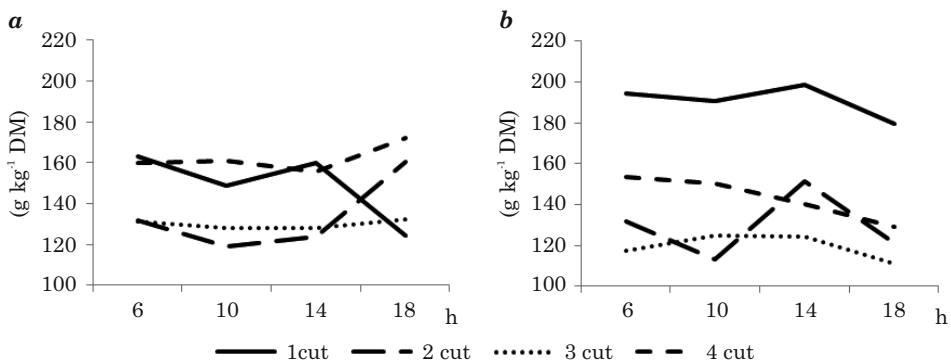

Fig. 2. Crude protein content in diploid (*a*) and tetraploid (*b*) perennial ryegrass herbage harvested at different times od the day

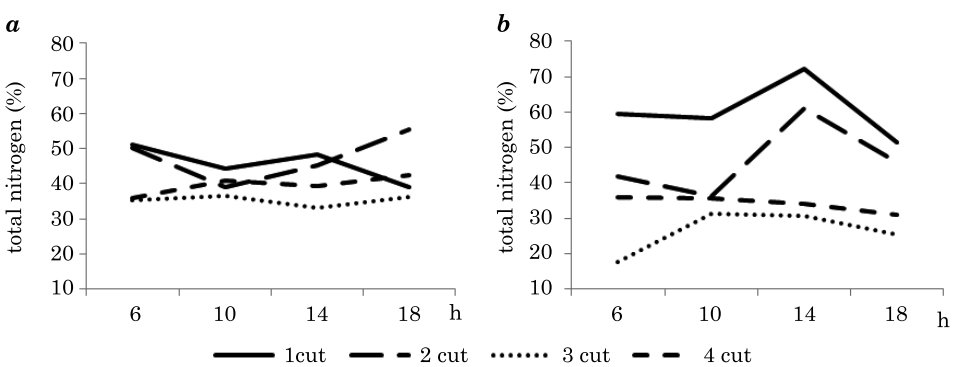

Fig. 3. Non-protein nitrogen content in diploid (*a*) and tetraploid (*b*) perennial ryegrass herbage harvested at different times od the day

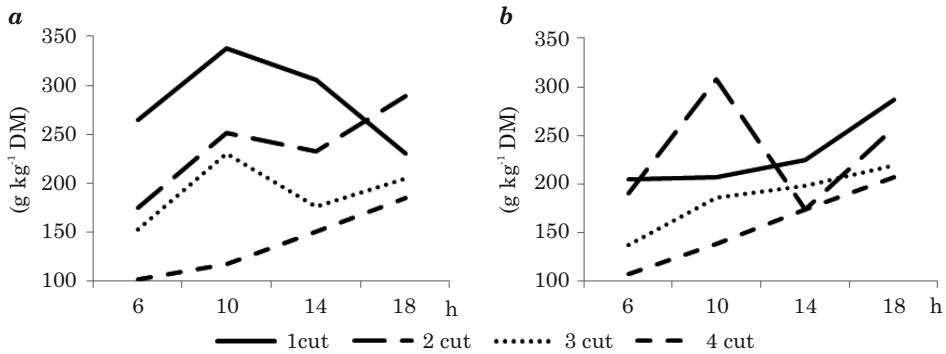

Fig. 4. Water soluble carbohydrate (WSC) content in diploid (*a*) and tetraploid (*b*) perennial ryegrass herbage harvested at different times od the day

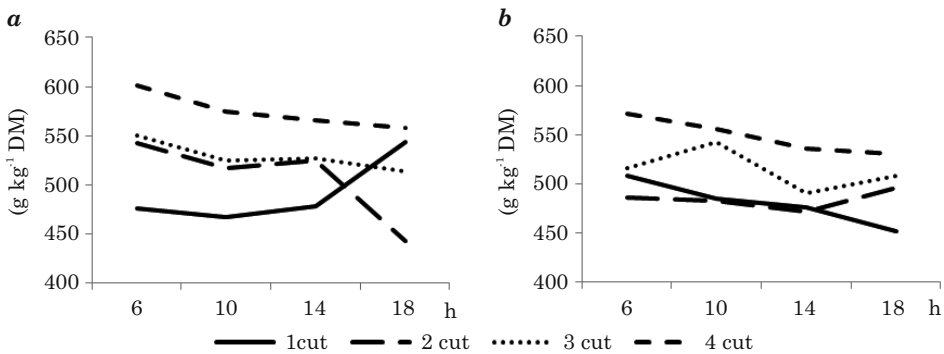

Fig. 5. aNDF content in diploid (*a*) and tetraploid (*b*) perennial ryegrass herbage harvested at different times od the day

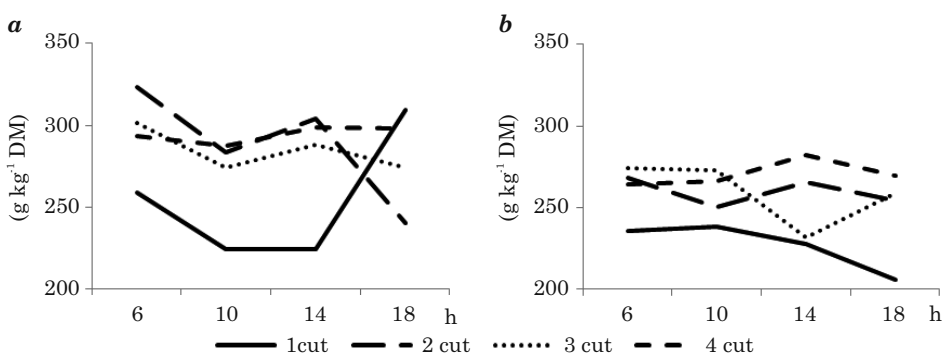

Fig. 6. ADF content in diploid (*a*) and tetraploid (*b*) perennial ryegrass herbage harvested at different times od the day

protein (CP) and carbohydrate fractions was confirmed. Also, interactions between these factors in relation to the analyzed components were confirmed. The diploid cultivar has a greater amount of DM, WSC and aNDF ($P < 0.01$) and lesser CP and ADF ($P < 0.01$) compared to the tetraploid cultivar (Table 1). The dry matter content in herbages from the fourth swath (IV) was higher in comparison with swaths I, II and III ($P < 0.01$). The dry matter of herbage from the first swath (I) was characterized by the highest concentration of WSC and the least content of aNDF ($P < 0.01$). In each following swath, the WSC content significantly decreased ($P < 0.01$) while aNDF increased ($P < 0.01$). The content of ADF in the herbage from swaths II and III was similar and higher ($P < 0.01$) than in swath I but smaller ($P < 0.01$) compared to swath IV. Herbage from the particular swaths differed in the CP content ($P < 0.01$). In both varieties of perennial ryegrass the content of CP in herbage from from swaths I and IV was higher than in swaths II and III. The content of non-protein nitrogen (NPN) decreased from the first to the last swath, and in swaths III and IV was lower than in swaths I ($P < 0.01$) and II ($P < 0.05$). The effect of the harvest time on the NPN content was not confirmed. However, some impact of the harvest time on DM, CP and carbohydrate fractions ($P < 0.01$) has been proven, but it was differentiated depending on a cultivar, a swath and or both of these factors simultaneously, as evidenced by the interactions $G \times H$, $P \times H$, $G \times P \times H$, ($P < 0.01$). Perennial ryegrass harvested at 6:00 was characterized by a lower mean content of DM and WSC and higher aNDF, ADF and CP compared to the other times, while the herbage harvested at 18:00 had the greatest content of DM and WSC.

Despite significant differences in the chemical composition of herbage, the influence of a cultivar and harvest time on DM degradability *in vitro* after 48 hours of incubation was not confirmed (Table 2).

Table 2

In vitro dry matter degradability (%) after 48-hours incubation of diplo- and tetraploid perennial ryegrass harvested at different times of the day

Genotype	Harvest time	Swath				SEM
		I	II	III	IV	
Diploid	6:00	84.1	76.7	76.0	75.0	2.08
	10:00	85.8	79.6	75.7	74.9	2.49
	14:00	87.0	78.4	75.8	78.2	2.45
	18:00	78.0	86.4	76.7	75.7	2.44
Tetraploid	6:00	84.6	79.6	80.8	77.7	1.45
	10:00	87.3	80.0	79.1	71.9	3.15
	14:00	84.6	83.5	81.9	70.8	3.18
	18:00	86.3	80.4	80.9	76.9	1.94

SEM – standard error of the mean

The largest differences in degradability occurred between swaths. The dry matter degradability of the diploid perennial ryegrass herbage from swaths I and II was greater than in swaths III and IV by 4 to 7 percentage points. The dry matter degradability of the tetraploid variety was the highest in the herbage from swath I and differed from that originating from swaths II and III by approx. 5 percentage points. The least degradability (about 11% points) was determined for the herbage from swath IV. The smallest variation occurred in the degradability of swath III, regardless of a variety. The dry matter degradability of swaths I and II of diploid varieties was strongly dependent on the harvest time.

A more varied and greater dry matter content in swath IV definitely resulted from the long regrowth time (6 weeks). The increase in the DM content between 6:00 and 10:00 occurs in all cuts of both varieties and can be explained by the drying up of dew, while the increase observed between 14:00 and 18:00 may have resulted from the accumulation of products of photosynthesis, which was confirmed by a parallel increase in the concentration of WSC. The lowering of the DM content in the afternoon was observed in concerned swaths I, II, III of both varieties (PxH), while the increase in DM in the afternoon was noticed only for the tetraploid cultivar (GxH) – Figure 1.

The mean CP content in the herbage from both varieties was similar, despite significant statistical differences. A higher CP content in tetraploid varieties was primarily observed in the first swath (Figure 2*b*). An approximately close content of CP was found by Krzywiecki, Kozłowski (2003). Taweel et al. (2005a) showed that the greatest content of CP is characteristic for swath I, with a reduction of its amount occurring in the following swaths. Similarly, while analyzing the impact of regrowth time (2-7 weeks) on the CP content in diploid and tetraploid perennial ryegrass Sun et al. (2010) found that the CP content decreased with a prolonged time of regrowth. In our study, the results for cuts II and III were consistent but contrary to the results cited above with respect to cut IV of both varieties.

Delgarde et al. (2000) analyzed the composition of herbage harvested in the spring and summer at different times of the day, noting a reduction of the CP content at the end of the day. In this study, the mean CP content decreased between 6:00-10:00 and 14:00-18:00. This course was characteristic for the first swath of both cultivars. The other swaths of both varieties were characterized by a different course of CP content changes during the day (Figure 2). This observation is confirmed by numerous significant interactions between the analyzed factors.

The contribution of NPN in CP was greater in the herbage from swaths I and II ($P < 0.01$), especially in the morning. During the day, there was a reduction in the contribution of NPN in the herbage from swath I, but more noticeable changes were observed in the tetraploid varieties of perennial ryegrass. However, the effects of the time of day or interactions were not confirmed statistically.

The mean WSC content in the herbage from all swaths was high and similar to that reported by Krzywiecki, Kozłowski (2003). Upward trends in the concentration of WSC in the herbage from subsequent cuts were shown by Taweel et al. (2006), who observed an increase in the WSC concentration from 12% in July to 20% in September. A higher amount of WSC in the diploid perennial ryegrass (first 3 cuts) observed in this research does not confirm the results of the cited authors, who showed a greater concentration of WSC in tetraploid cultivars. An analysis of the WSC content in subsequent swaths conducted by Miller et al. (2001) from March to August showed a greater content in May (up to 359 g kg^{-1} DM), a significantly lesser one in June (approx. 200 g), and the most effective harvest time turned out to be 18:00. Interactions between all the factors for the content of WSC resulted from the fact that in this study the least WSC content occurred in the herbage harvested at 6:00, and that was detected for 6 of the 8 analyzed ryegrass swaths (Figure 4). An exception was the first cut of the diploid cultivar, in which the least WSC was determined in the herbage harvested at 18:00 and the second cut of the tetraploid cultivar, where the least WSC was found in the herbage harvested at 14:00. The highest level of WSC for the diploid cultivar of swaths I, II and III was at 10.00 and for the tetraploid at 18:00. This may indicate that the most favorable harvest time for the diploid variety was at 10:00 while for the tetraploid one it was 18:00. An increasing concentration of WSC at 18:00 in tetraploid ryegrass was also found by Delgarde (2000) and Miller et al. (2001).

The observed increase in the mean content of fiber in herbage in the following swaths can be explained by a longer period of regrowth. An increase in the aNDF and ADF concentration of the diploid and tetraploid perennial ryegrass caused by the regrowth time (2-7 weeks) was also shown by Sun et al. (2010). The decreasing mean concentrations of aNDF and ADF in the afternoon were consistent with the observations of Cosgrove (2009) and Taweel (2005a), who analyzed the composition of ryegrass herbage from March to August between 6:00 and 20:00. However, in the present study, these trends were confirmed for the mean structural carbohydrate content (Table 1), and differences between cultivars and swaths were confirmed by interactions. A decrease of aNDF and ADF occurred in the herbage of the tetraploid perennial ryegrass from swath I between 14:00 and 18:00, while a significant increase was observed for the diploid (Figures 5, 6).

It needs to be emphasized that changes in the chemical composition during the day in aNDF, WSC and CP (particularly a higher ratio of WSC to CP) improve the efficiency of nitrogen utilization (Cosgrove et al. 2009), which can influence the degradability of organic matter.

The *in vitro* dry matter degradability obtained after 48 h of incubation can be regarded as high (Purwin et al. 2014). Despite the differences in the composition of herbage organic matter, differences in DM degradability have not been proven. The tetraploid herbage was characterized by greater *in vi-*

tro DM degradability, and this is confirmed by the results of Conaghana et al. (2008), who observed higher dry matter digestibility of tetraploid perennial ryegrass. In line with the trend of a decreasing mean WSC content and increase in the mean content of aNDF and ADF in subsequent swaths, the mean *in vitro* DM degradability decreased, which is consistent with the results obtained by Taweel et al. (2005b). The effect of the harvest time on the content of structural carbohydrates in the herbage from cut III was similar as on cut II. Also, a decreasing content of aNDF and ADF in the course of the day in the diploid herbage was observed. In the tetraploid variety, a greater content of aNDF and ADF was in the herbage harvested at 10:00. In the diploid cultivar of perennial ryegrass from swath I, the greatest aNDF and ADF content (Figures 5, 6) occurred at the same harvest times as the least WSC content (6:00 and 18:00).

The analysis of DM degradability of particular herbage samples (Table 2) showed a positive effect of the WSC content on DM degradability of only the herbage from cut IV, in which a lower WSC content and the least degradability of DM were found. The decrease in degradability could be affected by the longest period of regrowth (6 weeks). Although not statistically confirmed, differences in DM degradability of herbage between swaths were higher than between the diplo- and tetraploid varieties, which confirmed the results of Tas et al. (2006), who found a lower impact of genetic differentiation than a harvest time and weather conditions.

No effect of the harvest time on DM degradability with confirmed significant differences in the WSC content can be explained by the fact that DM degradability is positively correlated with the WSC content, but also highly digestible cell wall components (aNDF-ADF), where the release time is similar (Purwin et al. 2014). A large fraction of aNDF-ADF in experimental herbage (22 to 26% points of DM) could have a compensating effect on DM degradability. With a lesser amount of WSC, cellulolytic and hemicellulolytic microflora could develop more intensively. This is confirmed by Rooke et al. (1987), Bodarski, Krzywiecki (2001), who reported a reduction in the organic matter degradation of grass silage in the rumen after adding an additional amount of soluble carbohydrates. Chaves et al. (2006) found no effect of a higher structural carbohydrate content of herbage on the rate of DM degradation in rumen.

CONCLUSIONS

The effect of a cultivar and swath on the chemical composition of perennial ryegrass herbage was confirmed, but the differences in the chemical composition of cuts were greater than between the cultivars (diploid and tetraploid). The harvest time had significant and variable impact on the con-

tent of DM, CP and carbohydrate fractions depending on the genotype and swath. The large variation in the WSC content did not affect DM degradability of perennial ryegrass herbage harvested at different times of the day. The strong interaction observed between the analyzed factors indicates the need to choose harvest times carefully, depending on the genotype and swath. The results of *in vitro* DM degradability indicate that high value raw material of perennial ryegrass can be obtained at any time of the day. However, considering the suitability for making silage, the most favorable harvest time was 10:00 for the diploid cultivar (Bajka) and 18:00 for the tetraploid cultivar (Baronka).

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