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Cell wall fractions in the biomass of D*actylis glomerata* and F*estuca pratensis*

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The **purpose** of this paper is to determine the changes in the concentration of different fractions in the cell wall of *Dactylis glomerata* and *Festuca pratensis* growing on both mineral and organic soil with different harvesting times. This paper has drawn on two field experiments set up and carried out between 2010 and 2013 by the Research Centre for Cultivar Testing in Słupia Wielka. The experimental plots were sown with varieties of *Dactylis glomerata*: Niva, Tukan, Amila, Crown Royale and with varieties of *Festuca pratensis*: Limosa, Pasja, Anturka, Amelka (d. AND 1009). The experiment in Krzyżewo was set up on mineral soil. In Uhnin the experimental plots were located on peat meadow. The full exploitation of *Dactylis glomerata* varieties was due between 2012 and 2013, whereas for *Festuca pratensis* it was due between 2011 and 2012. In the experimental plots with the varieties of *Dactylis glomerata* the grass was harvested six times a year and chemical analysis of the biomass was done taking dry matter only from five cuts. The varieties of *Festuca pratensis* were harvested four times. Each year in the course of the experiment fresh and dry matter of each cut were weighed. The obtained results showed that the time of the harvest or cut for both of the grass species showed significantly different concentrations of the neutral detergent fiber fraction (NDF), acid detergent fiber fraction (ADF) and different concentrations of lignin and cellulose. For both of the grass species, plants harvested in the fourth cut had the highest content of different cell wall fractions. The location, that is the type of soil, affected the concentration of lignin (ADL), but only in the case of *Festuca pratensis*. A higher concentration of this polysaccharide was in the grass growing on organic soil.
Key words: NDF, ADF, ADL, cellulose, hemicellulose, grass

Introduction

According to Brzóska & Śliwiński (2011) cell wall components, as part of biomass, are mainly: neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) as well as cellulose and hemicellulose. Dissolving biomass in a neutral detergent allows determining NDF, an indicator of fiber content. To measure ADF, biomass is dissolved in acid, what is left is cellulose and lignin. Grygierzec (2012) says that cell walls of immature plants contain about 25% of cellulose and 60% of non-cellulosic polysaccharides in dry matter but only traces of lignin. In mature cells the content of cellulose is about 38% of cellulose, 43% of non-cellulosic polysaccharides, one of which is pectin, and 17% of lignin. The concentration of those substances can be used to determine the maturity of a plant. Fiber content is closely related to harvesting time, how mature a plant is when it is harvested but also to its morphological structure and to habitat conditions, in particular it is related to thermal conditions (Grygierzec, 2012.). One of the components of crude fiber is lignin, which fills the spaces of cell walls in the polysaccharide frame at the time when the cell is fully grown (Kucharska et al., 2007). Lignin is a three-dimensional polymer having a phenyl propane structure. In the cell wall lignin strengthens the bonds between the polysaccharide matrix and cellulose micro fibrils. Lignin and polysaccharide fill up the structure of the cell and prevent it from biochemical degradation and physical damage (Bach Knudsen, 1997). Hemicellulose, one of matrix polysaccharides (Vasiljevic et al., 2008) is part of the nutrients stored by the plant and, like cellulose, hemicellulose functions as supporting material in the cell wall, not digested by endogenous enzymes, but digested by enzymes produced by microorganism. Dissolution of hemicellulose depends on arabinose content. The higher the content of arabinose, the higher solubility of arabinoxylans is. Hemicellulose is a heteropolymer of hexoses and pentoses. These are stored between fibrils in the cell wall and they adhere to cellulose fibers (Bach Knudsen, 1997). In turn cellulose consists of long polymer chains of glucose. Like hemicellulose it is not digested by endogenous enzymes but is broken down by microorganisms (Choct,1997). Cellulose is the most abundant polymer in the plant cell wall (Annison, 1993). The cellulose structure consists of long chains of glucose linked by hydrogen bonds into micro fibrils. Adhering strongly to each other micro fibrils are interwoven which prevents water molecules from getting between the micro fibrils, making cellulose insoluble in water. Plants used to feed livestock can be particularly rich in cellulose and in mature plants cellulose is impregnated with lignin. This process, called lignification, is a result of cellular senescence (Bach Knudsen, 1997; Choct,1997).

The purpose of this paper is to determine the changes in the concentration of different fractions in the cell wall of *Dactylis glomerata* and *Festuca pratensis* growing on both mineral and organic soil with different harvesting times. The research tries to check whether the content of neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin (ADL), hemicelluloses and cellulose in the biomass of the studied grass species differs significantly in relation to the cut and the place where the grass grows.

Material and methods

This paper has drawn on two field experiments set up and carried out between 2010 and 2013 by the Research Centre for Cultivar Testing in Słupia Wielka. The experiment was conducted in two experimental stations: one in the Research Centre for Cultivar Testing in Krzyżewo and the other in the Experimental Stations for Variety Testing in Uhinin.
The experiment was arranged and conducted according to Research Centre for Cultivar Testing guidelines (Domański, 1998). The experimental plots were sown with varieties of *Dactylis glomerata*: Niva, Tukan, Amila, Crown Royale and with varieties of *Festuca pratensis*: Limosa, Pasja, Anturka, Amelka (d. AND 1009). The values of the characteristics presented in Tables 4-7 are the average of grasses varieties.
The plots were randomly selected, 1.5 meters wide and 6.67 m long, with an area of 10 m^2, grouped in blocks with four replications. They were separated by 1 meter pathways between blocks and with 0.5 meter pathways between sub-blocks. The pathways lay fallow. The experiment in Krzyżewo was set up on mineral soil. In Uhnin the experimental plots were located on peat meadow. Table 1 and 2 present soil characteristics and mineral fertilizers used.

Table 1 Soil conditions

Specification	Type of soil	
	Mineral soil	Peat soil
The value of soil according to IUNG	52	50
Agricultural value	5	1p
Type	P	PS
Texture	ls	-
pH	6.7	5.5

Symbols: 1p – good and very good permanent meadow, 5 – good quality rye soil; P – podsolic soil, PS – peaty soil, ls –loamy sand

In the research the amount of seeds of the grass sown varied depending on the variety and the location of the experiment. It was as follows (in kg·ha^{-1}): *Dactylis glomerata* – Tukan: 16.3; Amila: 17.5; CR: 18.8 (Krzyżewo) and 17.6 (Uhnin); Niva: 18.3, *Festuca pratensis* – Pasja: 28.7; Limosa:29.8 (Krzyżewo) and 27.1 (Uhnin); Anturka: 26.6; Amelka: 27.8 (Krzyżewo) and 27.9 (Uhnin).
The sowing dates for *Dactylis glomerata* were 22 April 2011 (Krzyżewo), 6 May 2011 (Uhnin) for *Festuca pratensis* 22 May 2011 (Krzyżewo) and 29 April 2011 (Uhnin).
In the year when the experiment was set up the grass was not harvested and only weeds were mowed. According to the guidelines of Research Centre for Cultivar Testing in Słupia Wielka, the full exploitation of *Dactylis glomerata* varieties was due between 2012 and 2013, whereas for *Festuca pratensis* it was due between 2011 and 2012. In the experimental plots with the varieties of *Dactylis glomerata* the grass was harvested six times a year and chemical analysis of the biomass was done taking dry matter only from five cuts. The varieties of *Festuca pratensis* were harvested four times. Each year in the course of the experiment fresh and dry matter of each cut were weighed.

Table 2 Mineral fertilizers used in the experiment with varieties of *Dactylis glomerata* and *Festuca pratensis*

Specification	*Dactylis glomerata* Fertilizers [kg·ha^{-1}]		*Festuca pratensis*	
	Mineral soil	Peat soil	Mineral soil	Peat soil
Nitrogen – N: before sowing, in consecutive years	270 245	80 192	80	80
Phosphorus – P_2O_5: before sowing, in consecutive years	90 80	100 100	80	80
Potassium – K_2O: before sowing, in consecutive years	90 130	100 110	100	100

Research Centre for Cultivar Testing in Słupia Wielka made those measurements available to be used in this paper. Immediately upon cutting, 1 kg sub-samples were placed on ice and transported to the laboratory whereupon the samples were dried at 60 ^oC. Dried samples were milled through a 1 mm steel mesh and analysed for dry matter (DM) and neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) fibre fraction (g kg^{-1} of dry matter). All determinations were made on the basis of near-infrared method by near infrared reflectance spectroscopy (NIRS) with the use of InfraAnalyzer 450. The NIRS calibrations used are based on the INRA data base. Moreover, basing on the equation developed by Van Soest et al. (1991) concerning the share in dry matter of individual fibre fractions, the content of cellulose and hemicellulose was calculated (g kg^{-1} of dry matter).
Statistical analysis of the data was done using Statistica 6.0 – 2001, with multifactorial analysis of variance. Tukey's test was used to find means that were significantly different from each other with level of significance $p \leq 0.05$.
Climatic conditions of the area where the experiment was carried out are typical for the 9th agricultural and climatic eastern part of Poland. The average annual air temperature varies from 6.7 to 6.9 °C and in the summer season the average 24 hour temperature is 15 °C. The growing season usually starts on 28 March, lasts till 30 October and is 200 to 220 days long. The average climatic water balance during the time of the experiment varied considerably according to the period and location. Annual rainfall ranges from 550 to 650 mm, with not frequent but recurrent rain. Water stress was mainly observed in spring while water deficit occurred in July (Radzka, 2014 a,b).
During the time of the experiment weather data were provided by the Meteorological and Hydrological Stations in Krzyżew and Uhnin. To determine temporal variation of meteorological parameters and their impact on plant growth Sielianinov's hydrothermal index was used with the month's classification according to Skowera and Puła (2004).

As it can be seen from table 3 space-time distribution of annual rainfall varied. April was a month of water stress only in 2011 in Krzyżewo (K = 0.86), whereas in May water deficit was noted in Uhnin in 2012 (K = 0.84). Every year in June there was enough rain both in Krzyżewo and Uhnin (K between 1.06 and 2.12). July was either extremely wet (Krzyżewo 2011 K = 3.9, Uhnin K = 3.03) or dry (Krzyżewo and Uhnin 2013). However, on the whole both July and August were rather dry whereas September and October happened to be extremely dry one year each with Sielianinov's hydrothermal index more that 4, (Krzyżewo 2013 and Uhnin 2012).

Table 3 Sielianinov's hydrothermal index (K) during the growing season in the years of the experiment in Krzyżewo and Uhnin

Month	Krzyżewo (mineral soil)			Uhnin (peat soil)		
	Year of experiment					
	2011	2012	2013	2011	2012	2013
IV	0.86 (s)	1.63 (dw)	2.50 (w)	1.39 (o)	1.06 (ds)	2.79 (bw)
V	1.64 (dw)	1.09 (ds)	1.80 (dw)	1.09 (ds)	0.84 (s)	2.87 (bw)
VI	1.06 (ds)	1.83 (dw)	1.53 (o)	2.12 (w)	1.92 (dw)	1.74 (dw)
VII	3.90 (sw)	1.55 (o)	1.08 (ds)	3.03 (sw)	0.81 (s)	0.92 (s)
VIII	1.15 (ds)	3.18 (sw)	0.89 (s)	0.79 (s)	1.25 (ds)	0.12 (ss)
IX	0.41 (bs)	0.40 (ss)	4.84 (sw)	0.21 (ss)	0.79 (s)	2.46 (w)
X	0.81 (s)	2.27 (w)	0.48 (bs)	1.27 (ds)	4.90 (sw)	0.46 (bs)

Note: (ss) – extremely dry, (bs) – very dry, (s) - dry, (ds) – quite dry , (o) - optymal, (dw) – quite wet, (w) - wet, (bw) – very wet, (sw) – extremely wet

Results and discussion

The research has shown (Tables 4-5) that for both *Dactylis glomerata* and *Festuca pratensis*, no matter what year it was, their dry matter from the second cut in Krzyżewo had the lowest NDF concentration. Moreover, it was found that concentration of *Dactylis glomerata* NDF was significantly affected by the time of the harvest and that there was a significant interaction between harvest time and other factors.

In both type of soil the highest NDF concentration was in the biomass from the fourth cut (on average 539 g·kg^{-1} of dry matter), while the lowest from the second cut (on average 486 g·kg^{-1} of dry matter). Like in the dry matter of *Dactylis glomerata*, the highest NDF concentration (543 g·kg^{-1} of dry matter) was found in the *Festuca pratensis* biomass from the fourth cut (Table 5). Type of soil (Tables 4-5) for both species of grass did not have a significant impact on the content of NDF in biomass. It is worth noting that the NDF content in the research was typical for grass biomass. In his research Grzelak (2010) found a similar average concentration of NDF in dry matter of dried meadow grass (470 g·kg^{-1} of dry matter). In turn, Jankowska-Huflejt & Wróbel (2008) analyzing hay in grasslands of organic farms found higher concentration of NDF, 594 g·kg^{-1} of dry matter. Moreover, in the research done by Sosnowski (2012) it turned out that the concentration of NDF in the dry matter of cocksfoot sampled from all three cuts was 530 g·kg^{-1} of dry matter on average.

As it can be seen from the tables 4 and 5 the concentration of the ADF fiber fraction in the analyzed plant material of *Dactylis glomerata* was closely related to a cut and its correlation with type of soil.

Table 4 The concentration of the fiber fraction NDF, ADF and ADL in the dry matter of *Dactylis glomerata* in relation to the cut and type of soil (average from all years)

Cut	Type of soil Mineral soil	Peat soil	Mean
NDF[g·kg^{-1} s.m.]			
I	478	516	497
II	477	496	486
III	518	516	517
IV	548	530	539
V	509	549	529
Mean	506	521	
$NIR_{0,05}$ for: cut x type of soil – 36; cut – 31; type of soil – statistically insignificant			
ADF [g·kg^{-1} of dry matter]			
I	286	305	295
II	295	294	295
III	325	306	316
IV	336	327	332
V	299	337	318
Mean	308	314	
$NIR_{0,05}$ for: cut x type of soil – 20; cut – 20; type of soil – insignificant			
ADL – Lignin [g·kg^{-1} of dry matter]			
I	31.2	37.9	34.5
II	30.1	34.9	32.5
III	36.6	39.6	38.1
IV	37.1	41.4	39.2
V	32.2	41.7	36.9
Mean	33.4	39.1	
$NIR_{0,05}$ for: cut x type of soil – 2.7; cut – 3.4; type of soil – insignificant			

The concentration of the ADF fiber fraction in the biomass of this species varied from 295 g·kg^{-1} of dry matter from the first and second cuts to 332 g·kg^{-1} of dry matter from the fourth cut. Biomass of *Festuca pratensis* had smaller differences between concentrations of ADF: from 301 g·kg^{-1} of first cut dry matter to 318 g·kg^{-1} of dry mater from the fourth cut.

Type of soil did not significantly affected concentration of this fraction in both *Dactylis glomerata* and *Festuca pratensis*. Jankowska-Huflejt & Wróbel (2008) found even smaller differences of the ADF fraction concentration (323 - 332 $g \cdot kg^{-1}$ of dry matter).

The concentration of ADL, lignin in *Dactylis glomerata* varied from 32.5 (the second cut) to 39.2 $g \cdot kg^{-1}$ of dry matter (the fourth cut). In the research no statistically significant correlation was found between the concentration of lignin in the dry matter of *Dactylis glomerata* and type of soil. However, the grass on peat soil showed a higher concentration of lignin (above 14%). On the other hand, there was a significant correlation between the factors themselves and between all the factors and the concentration of ADL in the dry matter of *Festuca pratensis*. Comparing cut averages it can be said that the lowest ADL was found in the second cut (35.4 $g \cdot kg^{-1}$ of dry matter) and the highest in the fourth cut (38.1 $g \cdot kg^{-1}$ of dry matter). The lowest content of lignin, no matter what cut, variety, year or type of soil, was in the grass biomass mineral soil, on average 35.1 $g \cdot kg^{-1}$ of dry matter. In the plant material taken from the organic soil, the concentration of the ADL fiber fraction was on average 38.5 $g \cdot kg^{-1}$ of dry matter.

There were no significant differences between the hemicellulose concentration in *Dactylis glomerata*, which was 202 $g \cdot kg^{-1}$ of dry matter (Table 6). The basic factors had no significant impact on the hemicelluloses concentration and there was no significant correlation between them. However *Festuca pratensis* (Table 7) had a varied concentration of this polysaccharide.

Table 5 The concentration of the fiber fraction NDF, ADF and ADL in the dry matter of *Festuca pratensis* in relation to the cut and type of soil (average from all years)

Cut	Type of soil Mineral soil	Peat soil	Mean
NDF[$g \cdot kg^{-1}$ of dry matter]			
I	476	510	493
II	474	492	483
III	514	516	515
IV	554	532	543
Mean	503	512	
$NIR_{0,05}$ for: cut x type of soil – 33; cut – 43; type of soil – insignificant			
ADF [$g \cdot kg^{-1}$ of dry matter]			
I	298	310	304
II	293	308	301
III	316	293	304
IV	319	316	318
Mean	306	310	
$NIR_{0,05}$ for: cut x type of soil – 12; cut – 16; type of soil – insignificant			
ADL - Lignin [$g \cdot kg^{-1}$ of dry matter]			
I	34.0	40.0	36.9
II	34.0	36.8	35.4
III	36.5	38.0	37.2
IV	37.0	39.1	38.1
Mean	35.1	39.0	
$NIR_{0,05}$ for: cut x type of soil – 7.1; cut – 2.5; type of soil – 3.2			

Table 6 The concentration of hemicellulose and cellulose in the dry matter of *Dactylis glomerata* in relation to the cut and type of soil (average from all years)

Cut	Type of soil Mineral soil	Peat soil	Mean
Hemicelullose [$g \cdot kg^{-1}$ of dry matter]			
I	192	212	202
II	182	201	192
III	193	209	201
IV	207	204	200
V	210	212	211
Mean	203	201	
$NIR_{0,05}$ for: cut x type of soil – insignificant; cut – insignificant; type of soil – insignificant			
Cellulose [$g \cdot kg^{-1}$ of dry matter]			
I	255	267	261
II	265	259	262
III	289	267	278
IV	299	285	292
V	267	296	282
Mean	275	275	
$NIR_{0,05}$ for: cut x type of soil – insignificant; cut – 20; type of soil – insignificant			

The most hemicellulose (235 $g \cdot kg^{-1}$ of dry matter) was in the dry matter of the fourth cut on mineral soil. The lowest amount (178 $g \cdot kg^{-1}$ of dry matter) was in the first cut also on mineral soil. While analyzing type of soil, disregarding other factors, there was no significant influence of the place, with the higher concentration of hemicellulose in the biomass of *Festuca pratensis* on peat soil (on average 202 $g \cdot kg^{-1}$ of dry matter) than on mineral soil (on average 197 $g \cdot kg^{-1}$ of dry matter).

The concentration of cellulose in the studied grass species significantly varied only in relation to different cuts. This concentration was from 255 to 299 $g \cdot kg^{-1}$ of dry matter in the case of *Dactylis glomerata* and from 255 to 282 $g \cdot kg^{-1}$ of dry matter in the case of *Festuca pratensis*.

Table 7 The concentration of hemicellulose and cellulose in the dry matter of *Festuca pratensis* in relation to the cut and type of soil (average from all years)

Cut	Type of soil Mineral soil	Peat soil	Mean
Hemicellulose [g·kg^{-1} of dry matter]			
I	178	200	189
II	181	182	182
III	199	223	211
IV	235	214	225
Mean	197	202	
$NIR_{0,05}$ for: cut x type of soil – 18; cut – 14; type of soil – insignificant			
Cellulose [g·kg^{-1} of dry matter]			
I	264	270	267
II	259	272	266
III	279	255	267
IV	282	276	279
Mean	271	271	
$NIR_{0,05}$ for: cut x type of soil – insignificant; cut – 14;. type of soil – insignificant			

Conclusions

The time of the harvest or cut for both of the grass species showed significantly different concentrations of the neutral detergent fiber fraction (NDF), acid detergent fiber fraction (ADF) and different concentrations of lignin and cellulose.
For both of the grass species, plants harvested in the fourth cut had the highest content of different cell wall fractions. The location, that is the type of soil, affected the concentration of lignin (ADL), but only in the case of *Festuca pratensis*. A higher concentration of this polysaccharide was in the grass growing on organic soil.
The concentration of all fractions of the cell wall in the dry matter of *Dactylis glomerata* and *Festuca pratensis* did not differ significantly.

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