

CHANGES IN THE CONTENT OF STRUCTURAL CARBOHYDRATES AND LIGNIN IN THE BIOMASS OF *Lolium multiflorum* (Lam.) AFTER APPLYING SLURRY WITH BIOPREPARATIONS AND WITH NPK

Beata Wiśniewska-Kadzajan[✉], Kazimierz Jankowski

Institute of Agriculture and Horticulture, Siedlce University of Natural Sciences and Humanities
B. Prusa 14, 08-110 Siedlce, Poland

ABSTRACT

Background. The research aimed at determining the effect of slurry supplemented with biopreparations UGmax and Humus Active and with NPK on the content of structural carbohydrates and lignin in the biomass of *Lolium multiflorum*.

Material and methods. A two-year field experiment in three replications with a completely randomized design was set up on an experimental plot of Siedlce University of Natural Sciences and Humanities. The cv. Dukat was tested. Plant material samples were taken from three cuts in each research year. Near infrared spectroscopy (NIRS) was used to determine the cellulose, hemicellulose and lignin contents.

Results. Using biopreparations and mineral NPK fertilizers increases cellulose, hemicellulose and lignin contents in the obtained biomass compared with the biomass fertilized only with slurry. When considering particular cuts, regardless of fertilization and research year, a slight decrease was observed in the content of cellulose and hemicellulose in line with the order of harvest. A higher content of cellulose and lignin and a lower content of hemicellulose in the harvested biomass of *Lolium multiflorum* was recorded in the second year.

Conclusion. The content of cellulose, hemicellulose and lignin in the biomass of *Lolium multiflorum* only underwent significant changes under the effect of the applied fertilization. Supplementing slurry with biopreparations and mineral NPK caused an increase in the content of cellulose, hemicellulose and lignin in the obtained biomass as compared with the biomass fertilized only with slurry.

Key words: cut, fertilization, *Lolium multiflorum*, structural carbohydrates

INTRODUCTION

An important assessment criterion of the feed value of forage grasses is the content of structural carbohydrates and lignin. Structural carbohydrates, also known as crude fiber, are carbohydrates that include cellulose and hemicellulose. An inherent component of structural carbohydrates is lignin and together they constitute a basic structural component of cell walls. Feed intake its digestibility and energy value depend on the content of cellulose, hemicelluloses

and lignin (Brzóska and Śliwiński, 2011; Baert *et al.*, 2012; Belanger *et al.*, 2013). Crude fiber has a large effect on the nutritional value of feed and on the health of animals (Rogulski, 2001).

Nitrogen fertilization is the most important agronomic factor determining yield and quality of forage grass (Tomic, 2012; Wróbel *et al.*, 2012; Ciepiela, 2014). The content of crude fiber depends on the plant species, cultivar and plant age (Domański, 2005; Grygierzec, 2012; Szkutnik *et al.*,

[✉] beata.wisniewska-kadzajan@uph.edu.pl, laki@uph.edu.pl

2012) as well as the time of harvesting and climatic conditions, especially the amount of rainfall, (Jankowska-Huflejt and Wróbel, 2010). According to Grzelak and Bocian (2009) high fiber content of green fodder and hay is a result of late cutting. Young plants contain little fiber, while along with the ageing of plants the fiber content increases. A certain amount of fiber in feed is desirable because it has a beneficial effect on the filling of the digestive system and inducing a sense of satiety. According to Łuczak and Rogalski (2004) the minimum content of crude fiber in animal feed is 10–15% DM. To maintain a high digestibility of nutrients and the energy value of given feeds the level of crude fiber should not exceed 28–30% DM (Dymnicka, 2001; Stachowicz, 2010).

The aim of the research was a determination of the effect of slurry as well as of the additional application of biopreparations UGmax and Humus Active and NPK on the content of structural components and lignin in the biomass of *Lolium multiflorum*.

MATERIAL AND METHODS

The two-year (2016–2017) field experiment was carried out in three replications in a completely randomized design on an experimental plot (52°10' N;

22°17' E) of Siedlce University of Natural Sciences and Humanities. The main factor was slurry applied separately and supplemented with mineral NPK and with biological preparations (trade names: UGmax and Humus Active). The experimental plots each had an area of 4.5 m². In the experiment the following plots were arranged: control (without fertilization), slurry, slurry + NPK; slurry + UGmax; slurry + Humus Active.

The test plant was forage grass of the species *Lolium multiflorum* cv. Dukat that was sown in autumn 2015 in accordance with the sowing standards. The slurry from milk cows used in the experiment was applied every year in a combined rate of 30 m³·ha⁻¹ that was divided into three equal parts (the first in the spring before start vegetation, the second and third not later than a week after harvesting the first and second cut). The content of dry matter (DM) in the slurry was 10%, and the ratio C:N (8:1). The amount of macronutrients were (g·kg⁻¹ DM): N – 33.0; P – 7.0; K – 13.3; MgO – 6.0 and Ca – 21.0, respectively.

Biopreparations were applied annually onto spring growth in 2016 and spring regrowth in 2017 at doses recommended by the producer i.e. UGmax – 0.6 dm³·ha⁻¹, Humus Active – 50 dm³·ha⁻¹ (their chemical composition is shown in Table 1).

Table 1. Composition of biological preparations

Preparation	Macronutrients, g·kg ⁻¹					Micronutrients, mg·kg ⁻¹					Microorganisms and others
	N	P	K	Ca	Mg	Na	Mn	Fe	Zn	Cu	
UGmax	1.2	0.2	2.9	–	0.1	0.2	0.3	–	–	–	lactic acid bacteria, photosynthetic bacteria
Humus Active	0.2	1.3	4.6	3.0	0.5	–	15	500	3	1	stable active humus with a population of gram-positive microorganisms

Mineral nitrogen fertilization in the form of (NH₄NO₃), phosphorus fertilization in the form of (Ca(H₂PO₄)₂), and potassium fertilization in the form of (KCl), were applied at the following rates: N – 100, P (P₂O₅) – 80, K (K₂O) – 120 kg·ha⁻¹.

Phosphorus fertilization was used once in spring, while nitrogen and potassium fertilization was at rates divided into three equal parts: the first in the spring before start of vegetation, the second and third after harvesting the first and second cuts (immediately after the application the slurry).

The experiment was set up on soil of a granulometric composition of slightly loamy sand, included in the order of anthropogenic soils, culturozems type, subtype hortisols (PTG, 2011). Soil content of organic carbon compounds (C_{org}) was on the level of $14.50 \text{ g}\cdot\text{kg}^{-1}$ DM, total nitrogen $1.36 \text{ g}\cdot\text{kg}^{-1}$ DM, the ratio of C:N was 10.6:1, and pH value 6.7. The content of the available forms of P ($170.00 \text{ mg}\cdot\text{kg}^{-1}$ DM) and Mg ($84.00 \text{ mg}\cdot\text{kg}^{-1}$ DM) in the soil indicated a high content, while the content of K ($114.00 \text{ mg}\cdot\text{kg}^{-1}$ DM) corresponded with an average content.

Hydrothermal conditions were elaborated based on meteorological data from the Hydrological and Meteorological Station in Siedlce. In order to determine time and space variation in meteorological elements and their effect on the course of plant growth, Sielianinov's hydrothermal coefficient was determined (Table 2) (Bac *et al.*, 1993). This coefficient was determined based on the monthly rainfall total (P) as well as the monthly total air temperatures (Σt) with the use of the formula: $K = P/0.1 \Sigma t$ (Skowera and Puła, 2004).

Table 2. Sielianinov's hydrothermal coefficient (K) in particular months of the growing seasons during the experiment

Year	Month							
	April	May	June	July	August	September	October	Mean
2015	1.36	1.87	1.64	0.59	1.92	0.64	0.12	–
2016	1.22	2.63	0.87	1.08	0.18	1.46	1.94	–
2017	2.88	1.15	1.08	0.45	0.96	1.92	1.90	–

$K < 0.5$ high drought; $0.51 - 0.69$ drought; $0.70 - 0.99$ – poor drought ; $K > 1$ no drought

Over the time of the experiment, three cuts of the cultivated grass were harvested in each growing season. After being cut from each plot the forage was weighed and 0.5 kg of green matter was sampled in order to determine the yield of the dry matter and to carry out chemical analyses. In the harvested plant material the content of cellulose, hemicellulose and lignin were determined with the use of the near infrared reflectance spectroscopy method (NIRS) with the use of a NIRFlex N-500 and with ready-made calibrations for dry feeds from the INGOT company. This method is described in detail in the Polish Standards PN-EN ISO 12099:2010 and in the literature (Burns *et al.* 2010; Reddersen *et al.*, 2012).

Statistical elaboration of the obtained results was conducted with the use of the analysis of variance for the three-factor experiment. Significance of the effect of experimental factors on the value of the studied traits was concluded based on the F Fisher-Snedecor

test and the $LSD_{0.05}$ value was verified with Tukey's test. Statistical program Statistica 6.0 (Statsoft-Team, 2011) was used for data analysis.

RESULTS

The cellulose content in the biomass of *Lolium multiflorum* depended on the type of fertilization (Table 3). The highest content of cellulose ($287.1 \text{ g}\cdot\text{kg}^{-1}$), on average from the research years, was observed in the biomass from the control. A very similar amount ($286.2 \text{ g}\cdot\text{kg}^{-1}$) was indicated in the biomass from the plot where slurry was applied along with the biopreparation UGmax. On the other fertilized plots the content of this parameter in the biomass of the cultivated grass was significantly lower, and the lowest level was observed in the biomass from the plot where only slurry was applied ($271.5 \text{ g}\cdot\text{kg}^{-1}$).

Table 3. Cellulose content in the dry matter of *Lolium multiflorum* ($\text{g}\cdot\text{kg}^{-1}$) in the two-year field experiment

Research year (B)	Cut (C)	Fertilization plots (A)					Mean
		O	*S	S + UGmax	S + HA	S + NPK	
2016	I	298.6	268.4	286.3	279.5	270.3	280.6
	II	280.5	253.9	276.5	290.3	268.1	273.9
	III	293.4	270.1	286.5	279.5	276.8	281.3
	Mean	290.8	264.1	283.1	283.1	271.7	278.6
2017	I	290.3	279.5	285.7	267.9	277.3	280.1
	II	275.8	289.6	292.4	286.4	287.3	286.3
	III	284.1	267.8	289.6	265.1	259.7	273.3
	Mean	283.4	278.9	289.2	273.1	274.8	279.9
Mean for both years		287.1	271.5	286.2	278.1	273.3	279.3
Mean from cuts							
	I	294.5	273.9	286.0	273.7	273.8	280.4
	II	278.2	271.8	284.5	288.4	277.7	280.1
	III	288.8	268.9	288.1	272.3	268.3	277.3
LSD _{0.05} for:	A = 16.5	B = ns	C = ns				
	A/B = 14.5	B/A = 9.70	A/C = ns				
	C/A = ns	B/C = 7.48	C/B = 9.27				

*S – slurry; S + UGmax – slurry + biopreparation UGmax; S + HA – slurry + biopreparation Humus Active; S + NPK – slurry + mineral fertilizers NPK

ns – not significant

The obtained contents of cellulose did not confirm any significant effect of the research years and cuts. However, the combination of the applied fertilization with the research years turned out to be significant as did research years with cuts.

Considering particular cuts, regardless of fertilization and research year, higher and comparable contents of cellulose were observed in cuts I and II: 280.4 and 280.1 $\text{g}\cdot\text{kg}^{-1}$, respectively, than in the biomass from cut III – 277.3 $\text{g}\cdot\text{kg}^{-1}$.

The mean contents of cellulose from fertilization and cuts in the first (278.6 $\text{g}\cdot\text{kg}^{-1}$) and second (279.9 $\text{g}\cdot\text{kg}^{-1}$) research year were similar.

The content of hemicellulose (Table 4) in the harvested biomass of *Lolium multiflorum* indicated variation according to the applied fertilization. The

largest amount of hemicellulose (mean from two years) was observed in the biomass from the plot fertilized with slurry supplemented with the biopreparation Humus Active (202.3 $\text{g}\cdot\text{kg}^{-1}$). The lowest content of hemicellulose was obtained in the control plot (172.7 $\text{g}\cdot\text{kg}^{-1}$).

The amount of hemicellulose from plots fertilized with slurry with the addition of biopreparations and NPK was significantly higher than in the plot fertilized only with slurry.

When analyzing particular cuts (regardless of fertilization and year), a higher content of hemicellulose in the biomass of the cultivated grass was observed in cut I and a lower content in cuts II and III.

The content of the studied parameter in the cultivated grass in the first research year was higher than in the second year.

The content of lignin (Table 5) in the biomass of *Lolium multiflorum* was influenced by the applied fertilization. The lowest lignin content (33.7 g·kg⁻¹) was observed in the biomass from the control plot. Application of slurry contributed to an increased content of lignin in the biomass. A statistically significant higher content of this parameter was

observed in biomass fertilized with slurry with the addition of the preparation UGmax (40.7 g·kg⁻¹), and with slurry with the addition of NPK (40.5 g·kg⁻¹).

When analyzing particular cuts (regardless of fertilization and year), it was found that the content of lignin in the biomass of cultivated grass was similar. Content of the studied parameter in the cultivated grass in the second research year (mean from fertilization and cuts) was higher (39.2 g·kg⁻¹) than in the first year (37.8 g·kg⁻¹).

Table 4. Hemicellulose content in the dry matter of *Lolium multiflorum* (g·kg⁻¹) in the two-year field experiment

Research year (B)	Cut (C)	Fertilization plots (A)					Mean
		O	*S	S + UGmax	S + HA	S + NPK	
2016	I	164.0	220.2	204.9	188.9	211.8	198.0
	II	153.3	181.5	212.3	203.4	157.5	181.6
	III	163.3	188.2	236.5	243.9	217.0	209.8
	Mean	160.2	196.6	217.9	212.1	195.4	196.5
2017	I	180.0	161.7	200.8	200.3	177.1	184.0
	II	201.9	175.9	204.7	209.2	185.1	195.4
	III	173.6	178.2	114.4	167.8	202.0	167.2
	Mean	185.2	171.9	173.3	192.4	188.1	182.2
Mean for both years		172.7	184.3	195.6	202.3	191.8	189.4
Mean from cuts							
	I	172.0	190.9	202.9	194.6	194.5	191.0
	II	177.6	178.7	208.5	206.3	171.3	188.5
	III	168.5	183.2	175.5	205.9	209.5	188.5
LSD _{0.05} for:	A = 41.7	B = ns	C = ns				
	A/B = 59.6	B/A = 39.8	A/C = ns				
	C/A = ns	B/C = ns	C/B = ns				

*S – slurry; S + UGmax – slurry + biopreparation UGmax; S + HA – slurry + biopreparation Humus Active; S + NPK – slurry + mineral fertilizers NPK
ns – not significant

Table 5. Lignin content in the dry matter of *Lolium multiflorum* (g·kg⁻¹) in the two-year field experiment

Research year (B)	Cut (C)	Fertilization plots (A)					Mean
		O	*S	S + UGmax	S + HA	S + NPK	
2016	I	31.8	29.8	40.2	38.9	37.6	35.7
	II	32.9	40.2	36.7	36.4	41.2	37.5
	III	39.7	41.8	40.3	36.1	43.5	40.3
	Mean	34.8	37.3	39.1	37.1	40.8	37.8
2017	I	35.6	45.9	41.2	44.9	39.6	41.5
	II	31.5	36.7	45.3	40.3	40.2	38.8
	III	30.9	35.6	40.6	39.5	40.5	37.4
	Mean	32.7	39.4	42.4	41.6	40.1	39.2
Mean for both years		33.7	38.4	40.7	39.3	40.5	38.5
Mean from cuts							
	I	33.7	37.9	40.7	41.9	38.6	38.6
	II	32.2	38.5	41.0	38.4	40.7	38.1
	III	35.3	38.7	40.5	37.8	42.0	38.9
LSD _{0.05} for:	A = 6.70	B = ns	C = ns				
	A/B = ns	B/A = ns	A/C = ns				
	C/A = ns	B/C = ns	C/B = ns				

*S – slurry; S + UGmax – slurry + biopreparation UGmax; S + HA – slurry + biopreparation Humus Active; S + NPK – slurry + mineral fertilizers NPK

ns – not significant

DISCUSSION

Cellulose is the most significant component of plant cell walls and occurs in larger amounts in young plants. In the process of lignification of the plant tissue, connected with ageing of its structures, there occurs incrustation of cellulose micelles with lignin (Van Soest *et al.*, 1991; Bach Knudsen, 1997).

In the conducted research the cellulose content in the biomass of *Lolium multiflorum* was significantly increased under the joint effect of slurry with biopreparations and with NPK as compared to the control.

Jankowska-Huflejt and Wróbel (2008) found that the content of cellulose in hay varied from 290.4 to

302.7 g·kg⁻¹ DM. That is comparable with the content of cellulose obtained in our research.

However, the study of Ciepiela (2014) showed that the biostimulator Kelpak and nitrogen (150 kg·ha⁻¹) significantly decreased cellulose content in the biomass of *Dactylis glomerata*. Their results also showed that the cellulose content in the biomass decreased with subsequent cuts.

According to Vasiljevic *et al.* (2008) an equally significant component in plant cell walls is hemicellulose.

The results of the conducted research showed that supplementation of slurry with biopreparations and mineral NPK led to an increased content of hemicellulose in the biomass of *Lolium multiflorum*

as compared with the biomass from the control plot and from that fertilized only with slurry. Contrary to this Ciepiela (2014) has indicated that the increasing of nitrogen doses caused a decrease in the content of hemicellulose in the yield of the cultivated grass.

In Ciepiela's (2014) research analysis of particular cuts indicated a decreased content of hemicellulose in cut II compared with cuts I and III. In our own research the hemicellulose content in the biomass of *Lolium multiflorum* was lower than that obtained by Ciepiela (2014), and was not significantly varied depending on the cut.

Research carried out by Truba *et al.* (2017) with the use of UGmax and Humus Active biopreparations without mineral fertilization showed an increase in the cellulose and hemicellulose content in the biomass of *Dactylis glomerata* and *Lolium perenne*, while their use together with mineral fertilization caused a decrease in these parameters in the biomass of the cultivated grass species.

According to Podkówka and Podkówka (2006) having an optimal content of lignin in the biomass is important, because in excess it limits the digestibility and energy value of the feed. The content of lignin indicates how advanced the process of plant lignification is (Kotlarz *et al.*, 2010).

In our studies the lignin content in the biomass of *Lolium multiflorum* was at the optimal level of 31.5–45.3 g·kg⁻¹ DM and showed a significant increase under the effect of fertilization.

In the research of Wróbel *et al.* (2013), carried out on a permanent meadow, the content of lignin was on the level of approximately 45 g·kg⁻¹, and after the application of cattle slurry the level of lignin decreased.

However, in the research of Barszczewski *et al.* (2010) the mean content of lignin in meadow sward was within the range of 37.6–52.9 g·kg⁻¹ DM and was higher than that found in the current research. In our own research the most lignin (mean from the years and fertilization) was observed in cut III and the lowest amount in cut II, although these differences were not significant, which is in contrast to the results of Barszczewski *et al.* (2010) who observed a higher content of this component in cut II compared to cuts I and III.

The content of the studied parameters in the harvested biomass of *Lolium multiflorum* was affected by weather conditions, which in both research years were unfavorable. In the first experimental year (2016), optimal thermal and moisture conditions occurred only in September with May being very humid, while June, July and August, that is months with the greatest effect on growth and development of plants, were quite dry, dry and extremely dry, respectively. In the second experimental year quite dry and dry periods prevailed from May to August, while optimal conditions were not observed in any month of the growing season.

CONCLUSIONS

1. The content of cellulose, hemicellulose and lignin in the biomass of *Lolium multiflorum* only underwent significant changes under the effect of the applied fertilization. Supplementing slurry with biopreparations and mineral NPK caused an increase in the content of cellulose, hemicellulose and lignin in the obtained biomass as compared with the biomass fertilized only with slurry.
2. Analysis of particular cuts, regardless of the applied fertilization and research year, indicated a slight decrease in the content of cellulose and hemicellulose in line with the order of harvest, while the content of lignin in every harvested cut was almost identical.
3. Higher contents of cellulose and lignin and a lower content of hemicellulose in the harvested biomass of *Lolium multiflorum* was recorded in the second year.

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ZMIANY ZAWARTOŚCI WĘGLOWODANÓW STRUKTURALNYCH ORAZ LIGNINY W BIOMASIE *Lolium multiflorum* (LAM.) POD WPŁYWEM STOSOWANIA GNOJOWICY Z BIOPREPARATAMI I NPK

Streszczenie

Badania miały na celu określenie wpływu gnojowicy uzupełnionej biopreparatami UGmax i Humus Active oraz NPK na zawartość węglowodanów strukturalnych i ligniny w biomacie *Lolium multiflorum*. Dwuletnie doświadczenie polowe w trzech powtórzeniach założono na obiekcie doświadczalnym Uniwersytetu Przyrodniczo-Humanistycznego w Siedlcach, w układzie całkowicie losowym. Rośliną testową była trawa pastewna gatunku *Lolium multiflorum* odmiany Dukat. W próbkach materiału roślinnego pobranych z trzech pokosów w każdym roku badań oznaczono zawartość celulozy, hemicelulozy i ligniny metodą spektroskopii odbiciowej w bliskiej podczerwieni (NIRS). Uzupełnienie gnojowicy biopreparatami i mineralnym NPK wywołało zwiększenie zawartości celulozy, hemicelulozy i ligniny w otrzymanej biomacie w odniesieniu do biomasy nawożonej wyłącznie gnojowicą. Rozpatrując poszczególne pokosy niezależnie od nawożenia i lat badań stwierdzono nieznaczne zmniejszenie zawartości celulozy i hemicelulozy wraz z kolejnością zbioru. Średnia z nawożenia i pokosów zawartość celulozy i ligniny w biomacie testowanej trawy w drugim roku była mniejsza niż w pierwszym, zaś zawartość hemicelulozy w biomacie z pierwszego roku była większa niż z drugiego.

Słowa kluczowe: *Lolium multiflorum*, nawożenie, pokos, węglowodany strukturalne