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VARIATION OF CARBOHYDRATES IN LEGUME-GRASS MIXTURES SUPPLIED BY MUSHROOM SUBSTRATE COMPOST AND COW SLURRY

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Abstract. The aim of the research was to assess the effect of mushroom substrate and slurry on the content of structural and non-structural carbohydrates in hybrid alfalfa mixtures with grasses. The three-year research was conducted in an experimental field between 2013 and 2015, with the following variables: (1) spent mushroom substrate (SMS) and cow slurry (CS), applied in different combinations; (2) three legume grass mixtures: orchard grass, perennial ryegrass, hybrid alfalfa (M1); orchard grass, hybrid alfalfa (M2); perennial ryegrass, hybrid alfalfa (M3). In each growing season, the mixtures were harvested three times. Plant material was used to determine dry matter content and the content of neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), cellulose, hemicellulose, lignin, total protein, crude ash, and crude fat by near infrared spectroscopy (NIRS), using the NIRFlex N-500 spectrometer and ready-to-use INGOT® calibration applications. Generally, mushroom substrate applied on its own increased the amounts of cellulose and hemicellulose in legume grass mixtures more than slurry. The most cellulose, the least hemicelluloses, and the highest degree of lignification were recorded in the mixture of ryegrass with alfalfa, while the degree of lignification was the smallest in the forage of alfalfa and orchard grass, which also contained the least cellulose but the most hemicellulose. On average, the highest amount of cellulose was in the biomass of the first harvest and the least in the third.

Key words: cellulose, hemicellulose, degree of lignification, non-structural carbohydrate.

INTRODUCTION

In the production of forage, its quality is as important as its yield. A source of very valuable green matter with components necessary for the nutrition of bovine animals, mixtures of legumes with grasses provide well-balanced foodstuffs (Gaweł 2008; Peyraud et al. 2009). A commonly used forage assessment criterion is the content of structural and non-structural carbohydrates and lignin (van Soest et al. 1991; Elgersma and Søegaard 2018). According to Truba et al. (2017), cellulose and hemicellulose are to a large degree digested by animals, cellulose much more slowly than hemicellulose, while lignin is not (Thomet et al. 2011). These polysaccharides limit the digestibility and energy value of crops (Das et al. 2015; Singh et al. 2018), and they reduce

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forage intake by animals. Not only their excessive intake but also their shortage in ruminant feed rations should be avoided (van Soest et al. 1991; Elgersma and Søegaard 2018).

Carbohydrate content in forage grass species varies to a large extent. As highlighted by Kozłowski and Swędrzyński (2001), this is the result of many biological, ecological, and anthropogenic factors, including plant growing methods. Rational fertilizer treatment is necessary in the cultivation of legume-grass mixtures. According to Jankowski and Malinowska (2019), it determines both the large yield and the desirable quality of forage necessary for proper ruminant nutrition.

Poland is one of the main producers of mushrooms in Europe, but it leads to the creation of large amounts of waste in the form of spent mushroom substrate (SMS) (Jankowski and Malinowska 2019). It is a serious problem for mushroom farmers who do not have a suitable area of farmland to use it. In turn, as many researchers have indicated (Guo and Chorover 2004; Søegaard and Weisbjerg 2007; Hackett 2015; Jankowski and Malinowska 2019; Coles et al. 2020), SMS is a potential source of a valuable organic fertilizer in agriculture, but the need to dispose of it poses a serious environmental problem. In accordance with the Regulation of the Minister of the Environment (2015), it is classified as waste with the possibility of agricultural use, to be processed by way of R10 Recovery. Research in this area has been carried out both on arable land (Guo and Chorover 2004; Hackett 2015; Coles et al. 2020) and on grassland (Jankowski et al. 2005; Ciepiela et al. 2007; Wiśniewska-Kadżajan 2013a, b; Paula et al. 2017), as well as in horticulture (Uzun 2004; Danai et al. 2012; Kuśmirek et al. 2012; Paula et al. 2017) and on lawns (Wiśniewska-Kadżajan 2013c). Taking into account animals nutrient requirements, Wiśniewska-Kadżajan (2013a, b) found that in forage fertilized with SMS there was optimum content of potassium and magnesium, with deficiency of calcium and sodium.

Another valuable and quick-release organic fertilizer is slurry, the nutrients of which are more absorbable to plants than those in manure (Sanchez and Gonzalez 2005; Christensen et al. 2009; Sørensen and Eriksen 2009; Noemi et al. 2020). Pig slurry application at the same nitrogen rate as that used for synthetic fertilizers can result in similar crop yields (Goss et al. 2013). A combination of slurry and mushroom substrate can increase the yield of plants as well as improve their nutritional value. Additionally, due to the larger number of microorganisms in slurry (Sanchez and Gonzalez 2005; Christensen et al. 2009), there may be an increase in the availability of nutrients both from the soil and from the organic materials introduced.

To the authors' knowledge, so far there have been no publications dealing with the above topic. Consequently, the aim of the research was to assess the effect of mushroom substrate and slurry applied to hybrid alfalfa mixtures with grasses on forage quality, especially on the content of structural and non-structural carbohydrates.

MATERIAL AND METHODS

Set up in the autumn of 2012 and ended in 2015, the three-year research was conducted in the experimental field of the Department of Grasslands and Landscape Architecture Development, University of Natural Sciences and Humanities in Siedlce, Poland. The experiment was replicated three times, with a split-plot arrangement and plots of 3 m^2 (1.5 m × 2 m) as experimental units. The total number of plots was 54.

In the experiment the main research variables were legume-grass mixtures, a nutrient-rich organic by-product, and a natural fertilizer, i.e. mushroom substrate and cow slurry, used separately and in various combinations. The experiment consisted of the following units:

1) control (no treatment);
2) spent mushroom substrate 30 $t \cdot ha^{-1}$ (SMS);
3) cow slurry 60 $m^3 \cdot ha^{-1}$ (CS);
4) mushroom substrate 10 $t \cdot ha^{-1}$ + cow slurry 60 $m^3 \cdot ha^{-1}$ (SMS_{10} + CS_{60});

5) mushroom substrate 20 t·ha^{-1} + cow slurry 40 m^3·ha^{-1} (SMS_{20} + CS_{40});
6) mushroom substrate 30 t·ha^{-1} + cow slurry 20 m^3·ha^{-1} (SMS_{30} + CS_{20}).

Because of increasing drought intensity, it is advisable to select plants more resistant to water shortage. Such species include, among others, hybrid alfalfa. Although it requires a significant amount of water for optimal development, it is able to survive long-term droughts and to recover very quickly thanks to a very well-developed root system that goes very deep into the soil profile. Therefore, this species is recommended for growing on its own and in mixtures with grasses. Because of the climatic conditions, orchard grass and ryegrass are most often cultivated in Poland. Therefore, mixtures of these three species in various modifications were used in the present experiment.

In the experiment three plant species were included: *Medicago* x varia Martyn (alfalfa hybrid) var. Tula, *Dactylis glomerata* (orchard grass) var. Bora, and *Lolium perenne* (perennial ryegrass) of the durable Info variety. These species were grown as three legume-grass mixtures with the same percentage share of each component:

– *Dactylis glomerata*, *Lolium perenne*, *Medicago* x varia Martyn (M1);
– *Dactylis glomerata*, *Medicago* x varia Martyn (M2);
– *Lolium perenne*, *Medicago* x varia Martyn (M3).

Taking into account different germination capacity, the sowing rate of plants grown on their own was as follows: *Medicago* x varia Martyn – 23 kg; *Dactylis glomerata* – 21 kg; *Lolium perenne* – 31 kg·ha^{-1}.

Mushroom substrate was applied once at the start of the experiment and mixed with a 20–25 cm layer of the soil. Slurry was used each year in three doses. On plots with lower amounts of cow slurry additional quantities of water were used so that the amount of liquid was the same on each unit. Each dose of slurry was applied before each growth cycle (mid-April, end of May, end of July). The chemical composition of organic materials is presented in Table 1.

Table 1.Concentration of selected macronutrients (g·kg^{-1}DM) in mushroom substrate and slurry

Nutrient	Spent mushroom substrate	Cow slurry
N	24.50	48.00
P	9.50	12.64
K	13.20	43.16
Ca	58.20	30.75
DM (%)	30	10

The experiment was conducted on soil of the anthropogenic order (A), the type of culture-earth soils (AK) and the subtype of hortisols (AKho). The soil developed from heavy loamy sand, with a layer of light loamy sand deep underneath (pgm and pgl) (Polish Soil Classification 2019). According to the chemical analysis, the content of absorbable forms of phosphorus (170.00 mg·kg^{-1} of dry matter – DM) and magnesium (84.00 mg·kg^{-1} DM) in the soil was high. However, absorbable forms of potassium (114.00 mg·kg^{-1} DM) were within the medium content. Carbon content in soil organic compounds (C_{org}) was 13.50 g·kg^{-1} DM, with nitrogen content of 1.30 g·kg^{-1} DM, the C to N ratio of 10.4 : 1, and pH of 6.8. The amounts of absorbable forms of nitrogen were as follows: N-NH_4^+ 20 mg·kg^{-1}; N-NO_3^- 245 mg·kg^{-1} (PN-R-04028, 1997).

DETERMINATION METHODS

During each growing season all the mixtures were harvested three times (beginning of May, mid-July, end of September). Fresh matter from each plot was weighed, and a sample of 0.6 kg was collected. It was used to determine dry matter content and to perform chemical analysis to measure cellulose, hemicellulose, lignin, total protein, crude ash, and crude fat content. It was done using near infrared spectroscopy (NIRS) with the NIRFlex N-500 spectrometer and ready--to-use INGOT® calibration applications. INGOT® is a set of universal NIR calibrations (adapted to the NIRFlex N-500 data format) for the analysis of raw materials and finished products, e.g. grass. Non-structural carbohydrate amounts were calculated following Virkajärvi et al. (2012): non-structural carbohydrates = 1000 – (total protein + crude ash + crude fat + cellulose + hemicelluloses + lignin). The degree of lignification was determined with the formula: ADL/NDF x 100 (Danai et al. 2012). Sielianinov's hydrothermal coefficient was calculated in order to determine temporal variation of meteorological conditions.

STATISTICAL ANALYSIS

The three-factor analysis of variance (ANOVA) for the experimental split-plot design was used. The following research variables were considered in the experiment: (A) – treatments (6 levels), (B) – mixtures (3 levels), and (C) – growth cycles / years (3 levels). The results were statistically processed using a three-factor analysis of variance according to the mathematical model:

$$y_{ijlp} = m + a_i + g_j + e_{ij}^{(1)} + b_l + c_p + ab_{il} + ac_{ip} + bc_{lp} + abc_{ilp} + e_{ijlp}^{(2)}$$

where:
y_{ijlp} – the value of the variable for the *i*-th level of factor A and *p*-th level of factor C for the *j*-th replicate;
m – the mean of research;
a_i, b_l, c_p – the effects of factors; *a* – treatments, *b* – mixtuers, *c* – growth cycles/years;
g_j – the effect of the *j*-th replicate;
ab_{il}, ac_{ip}, bc_{lp} – the effects of the interaction of two factors;
abc_{ilp} – the effect of the interaction of three factors;
e_{ij}, e_{ijlp} – the effect of random factor;
$i = 1, 2, \ldots, b$; *b* – the number of levels of factor B;
$p = 1, 2, \ldots, c$; *c* – the number of levels of factor C.

In this experiment, the fertilizer combinations and legume-grass mixtures were the fixed effects, but the years and error the random effects.

The Fisher-Snedecor test was carried out to determine the significance of the effects of experimental factors on the parameters tested. Tukey's test at $p < 0.05$ was used to compare means. In all tables and figures, different letters were included in the same row or column to indicate significant differences between treatments. Error bars were added to the figures. All the calculations were performed with the Statistica StatSoft 10.0.

RESULTS AND DISCUSSION

Weather conditions

Optimal temperature and moisture conditions (Table 2) were only in April 2014 and in September 2015. In the remaining months of the growing period the weather was not favourable, varying from extremely dry in August 2015 to extremely wet in May 2013. Throughout the experiment the best weather conditions were at the beginning of each growing period. It can be concluded that 2015 was the most unfavourable for plants, when, apart from May and the end of the growing period, the weather ranged from moderately dry to extremely dry.

Table 2. The value of Sielianinov's hydrothermal coefficient (K) in the growing season

Year	Month						
	April	May	June	July	August	September	October
2013	2.56 (sw)	3.07 (ew)	2.11 (w)	0.84 (d)	0.78 (d)	2.53 (sw)	0.60 (sd)
2014	1.36 (o)	1.87 (mw)	1.64 (mw)	0.59 (sd)	1.92 (mw)	0.64 (sd)	0.12 (ed)
2015	1.22 (md)	2.63 (sw)	0.87 (d)	1.08 (md)	0.18 (ed)	1.46 (o)	1.94 (dw)

K ≤ 0.4 extreme drought (ed), 0.4 < K ≤ 0.7 severe drought (sd), 0.7 < K ≤ 1.0 drought (d), 1.0 < K ≤ 1.3 moderate drought (md), 1.3 < K ≤ 1.6 optimal (o) 1.6 < K ≤ 2.0, moderately wet (mw) 2.0 < K ≤ 2.5 wet (w), 2.5 < K ≤ 3.0 severely wet (sw), K > 3.0 extremely wet (ew).

Cellulose

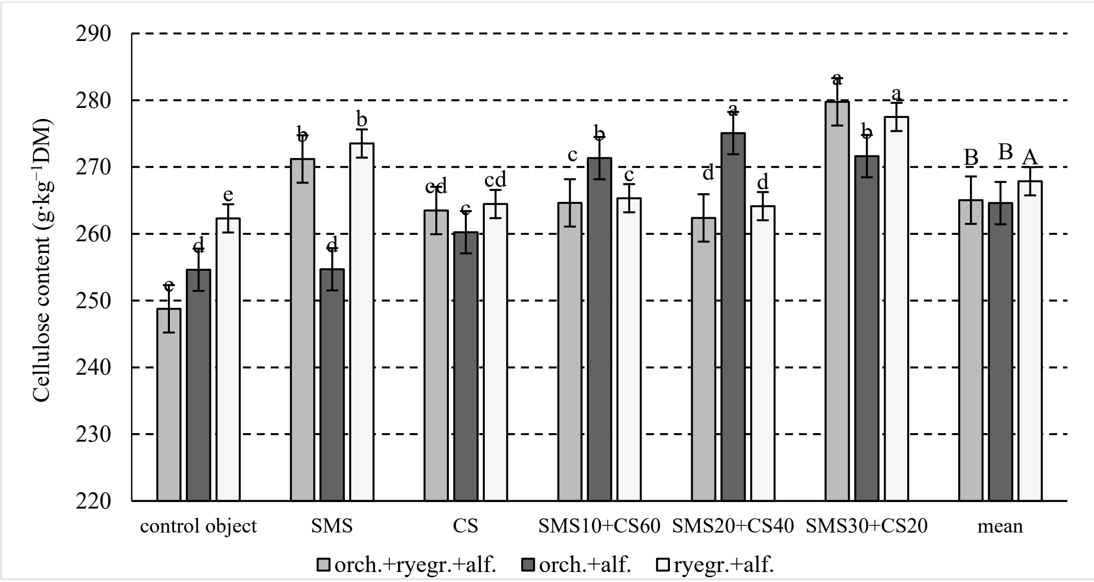
Treatment affected cellulose content in the plant material (Table 3, Fig. 1). Its highest amount (276.29 g·kg^{-1}) was found in plants treated with 30 t of mushroom substrate compost and 20 m^3 of slurry, and the lowest (255.23 g·kg^{-1}) in control plants (Fig. 1). These values were similar to those presented by Jankowska-Huflejt and Wróbel (2008) and by Truba et al. (2017). Cellulose content in plants treated with other fertilizer combinations (Table 3) did not very much, ranging from 262.72 to 267.20 g·kg^{-1}, but the statistical analysis results indicated significant differences between all of them. According to Salama and Nawar (2016), grass contained significantly more cellulose (345 g·kg^{-1}) than legumes or grass-legume mixtures.

Table 3.The effect of organic fertilizer combinations on cellulose content in legume-grass mixtures across growing seasons (g·kg^{-1} DM)

Mixture (C)	Year (B)	Treatment (A)						Mean
		0	SMS	CS	$SMS_{10} + CS_{60}$	$SMS_{20} + CS_{40}$	$SMS_{30} + CS_{20}$	
M1 – orchard grass + ryegrass + alfalfa	2013	248.11	269.17	260.27	254.46	266.35	291.23	264.93
	2014	244.89	265.03	265.46	258.06	250.09	271.31	259.14
	2015	253.30	279.37	264.73	281.37	270.70	276.74	271.04
M2 – orchard grass + alfalfa	2013	246.12	252.94	259.94	279.05	273.14	287.63	266.47
	2014	260.33	246.94	261.40	269.51	270.03	256.51	260.79
	2015	257.41	264.16	259.36	265.46	282.08	270.74	266.54
M3 – ryegrass + alfalfa	2013	265.11	275.06	266.39	267.53	260.35	265.27	266.62
	2014	255.11	268.09	262.80	263.13	266,77	271,00	264.48
	2015	266.69	277.42	264.18	265.31	265.28	296.26	272.52
Mean effect of treatment								
		255.23^{e}	266.46^{c}	262.72^{d}	267.10^{b}	267.20^{b}	276.29^{a}	265.83
Mean effect of the growing season								
2013		253.11^{e}	265.72^{c}	262.20^{d}	267.02^{b}	266.61^{b}	281.37^{a}	266,01^{B}
2014		253.45^{e}	260.02^{d}	263.22^{b}	263.56^{b}	262.30^{c}	266.27^{a}	261.47^{C}
2015		259.14^{f}	273.64^{b}	262.76^{e}	270.72^{d}	272.69^{c}	281.25^{a}	270.03^{A}

Different lowercase letters in the same row or different uppercase letters in the same column indicate significant differences between treatments.

According to Choct (1997), cellulose consists of combined glucose molecules, Samman and Annison (1993) points out that it is the most significant component of plant cell walls. According to Bach Knudsen (1997), the way cellulose is structured prevents it from being penetrated by water molecules, which has a decisive impact on the fact that it is insoluble in water. Cellulose is found in large quantities in young plants, while in older ones cell walls are impregnated with lignin, and the process of tissue lignification accelerates with the aging of plant structures. According to Jankowska-Huflejt and Wróbel (2008), cellulose content is the lowest in pasture green matter, ranging from 249.8 to 271.0 g·kg^{-1} DM, and it is similar both in meadow green matter (from 287.7 to 299.7 g·kg^{-1} DM) and in hay (from 290.4 to 302.7 g·kg^{-1} DM).



orch.+ryegr.+alf. – orchard grass + ryegrass + alfalfa (M1); orch.+alf. – orchard grass + alfalfa (M2); ryegr.+alf. – ryegrass + alfalfa (M3)

Fig. 1. The effect of organic fertilizer combinations (SMS and CS) on cellulose content (g·kg^{-1} DM) in legume-grass mixtures across harvests (average values of all growing seasons). Different lowercase letters for each mixture indicate significant differences between treatments. Vertical bars indicate the standard error

Cellulose content in plant material statistically varied across growing seasons (Table 3); its highest amount was in the third one (270.03 g·kg^{-1}) and the lowest in the second (261.47 g·kg^{-1}). The same tendency was also recorded for individual legume-grass mixtures. These results clearly indicated a very strong relationship between cellulose content and fluctuations in annual weather conditions. In the third year, in most months of the growing period (Table 2), there was a drought with different severity. These conditions resulted in faster lignification of cell walls, thus increasing the amount of cellulose in plants.

It was found that cellulose content in plant material was also dependent on the harvest (Table 4). Its greatest amount, as an average across treatments and legume-grass mixtures, was in plants harvested in the first cut (272.14 g·kg^{-1}) and the smallest in the third (256.58 g·kg^{-1}). This was confirmed by the studies of Truba et al. (2017), in which the highest amount of cellulose, average for orchard grass and perennial ryegrass, was in the first harvest (258.3 g·kg^{-1}) and the lowest in the third (237.9 g·kg^{-1}). Similarly, Ciepiela (2014) found that cellulose content in orchard grass decreased with subsequent harvests, being highest in the first one (335.1 g·kg^{-1} DM) and lowest in the third (311.3 g·kg^{-1} DM). In the present studies (Table 4) the same relationship was also recorded in the mixture of orchard grass with ryegrass and alfalfa and in the one with orchard grass and alfalfa. In the first growth cycle grass developed fewer leaves, and, in consequence, in the first harvest an increase in the content of cellulose was recorded. Ciepiela

(2014) found that the share of leaf blades in the orchard grass yield from the first harvest was 42%, but it increased to 75% in the third.

Table 4. The effect of organic fertilizer combinations on cellulose content in legume-grass mixtures across harvests (g·kg⁻¹ DM)

Mixture (C)	Harvest (B)	Treatment (A)						Mean
		0	SMS	CS	$SMS_{10} + CS_{60}$	$SMS_{20} + CS_{40}$	$SMS_{30} + CS_{20}$	
M1 – orchard grass + rye-grass + alfalfa	I	267.56	276.05	265.91	273.38	267.93	290.08	273.48
	II	241.15	276.91	263.02	264.85	257.53	287.16	265.10
	III	237.60	260.62	261.56	255.66	261.69	262.03	256.52
M2 – orchard grass + alfalfa	I	256.67	253.25	264.01	271.49	275.43	302.38	270.54
	II	255.44	263.33	255.44	276.08	282.64	271.13	267.23
	III	251.74	247.47	261.96	266.46	267.17	241.37	256.02
M3 – ryegrass + alfalfa	I	269.23	288.91	266.31	246.82	268.11	295.09	272.41
	II	262.66	273.97	280.02	289.02	260.16	278.36	274.03
	III	255.04	257.69	247.04	260.12	264.15	259.08	257.19
Mean effect of harvest								
I		264.48^{d}	272.73^{b}	265.41^{d}	263.90^{d}	270.49^{c}	295.85^{a}	272.14^{A}
II		253.09^{f}	271.40^{c}	265.93^{e}	276.65^{b}	266.78^{d}	278.88^{a}	268.78^{B}
III		248.13^{e}	255.26^{d}	256.85^{c}	260.75^{b}	264.33^{a}	254.16^{d}	256.58^{C}

Different lowercase letters in the same row or different uppercase letters in the same column indicate significant differences between treatments

The content of cellulose varied across mixtures (Fig. 1). Its greatest amount was in the forage of ryegrass with alfalfa (267.87 g·kg⁻¹) and the smallest in orchard grass with alfalfa (264.60 g·kg⁻¹), with that difference being statistically significant. Studying the interaction between organic fertilizer treatments and legume-grass mixtures, it was observed that the dose of 30 t of mushroom substrate compost and 20 m^3 of slurry increased cellulose content in the mixture of orchard grass with ryegrass and alfalfa (279.76 g·kg⁻¹) and in the one with ryegrass and alfalfa (277.50 g·kg⁻¹). The lowest amount of cellulose was recorded in control plots.

Hemicellulose

Hemicellulose content in forage (Table 5) varied and ranged between 28.80 and 84.68 g·kg⁻¹. Its amount was dependent, among others, on the treatment. As a mean effect of treatment (Table 5, Fig. 2), the highest was in the forage treated with mushroom substrate compost on its own (72.16 g·kg⁻¹) and the lowest after applying 10 t of compost with 60 m^3 of slurry (59.24 g·kg⁻¹). These differences were statistically significant.

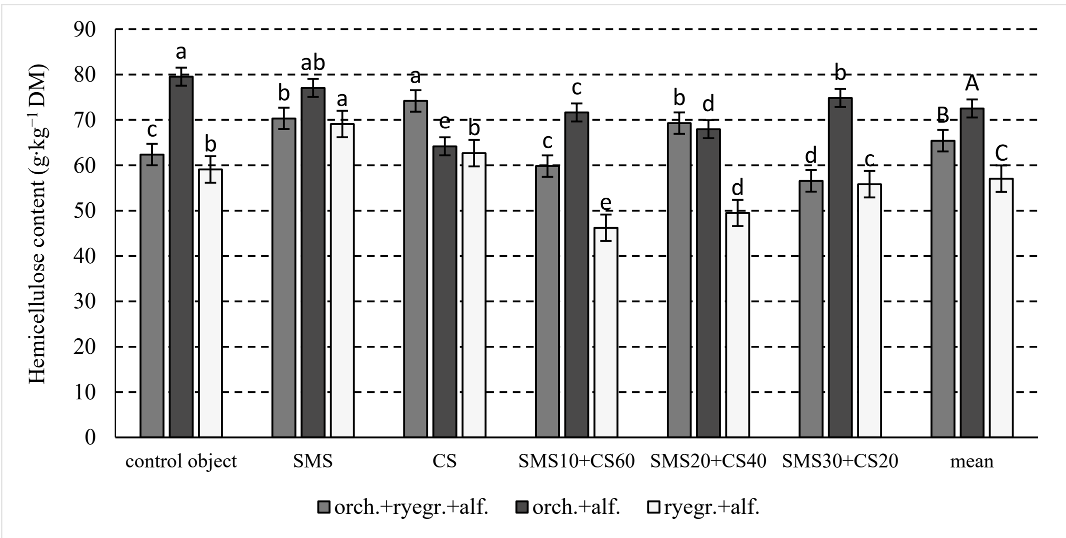
Ciepiela (2014) found that an increasing dose of nitrogen reduced hemicellulose content. The results of the present studies indicated that by reducing the dose of mushroom substrate compost and increasing the dose of slurry, hemicellulose content in the forage increased although the differences were not statistically significant.

Hemicellulose is an important component of plant cell walls (Vasiljevic et al. 2008), and, according to Bach Knudsen (1997), it is also a storage product, accompanied by cellulose. Hemicellulose is mainly composed of pentoses and hexoses and is stored in cell wall structures, adjacent to cellulose fibres.

Table 5.The effect of organic fertilizer combinations on hemicellulose content in legume-grass mixtures across growing seasons (g·kg^{-1} DM)

Mixture (C)	Year (B)	Treatment (A)						Mean
		0	SMS	CS	SMS_{10} + CS_{60}	SMS_{20} + CS_{40}	SMS_{30} + CS_{20}	
M1 – orchard grass + ryegrass + alfalfa	2013	73.67	73.66	71.61	40.30	84.35	28.80	62.06
	2014	61.34	61.23	75.82	63.67	54.56	57.87	62.41
	2015	52.09	76.12	75.10	75.50	68.95	83.03	71.80
M2 – orchard grass + alfalfa	2013	80.11	71.82	56.59	73.04	49.50	69.73	66.80
	2014	77.67	84.68	63.01	71.07	79.13	76.38	75.32
	2015	80.80	74.63	72.97	70.85	75.21	78.37	75.47
M3 – ryegrass + alfalfa	2013	73.22	76.60	57.21	31.29	39.77	63.25	56.89
	2014	45.89	61.35	55.99	45.52	42.95	32.80	47.42
	2015	58.13	69.30	74.81	61.90	65.73	71.41	66.88
Mean effect of treatment								
		66.99^{b}	72.16^{a}	67.01^{b}	59.24^{d}	62.24^{c}	62.41^{c}	65.00
Mean effect of the growing season								
	2013	75.67^{a}	74.03^{a}	61.81^{b}	48.21^{e}	57.87^{c}	53.93^{d}	61.92^{B}
	2014	61.63^{bc}	69.09^{a}	64.94^{b}	60.09^{c}	58.88^{cd}	55.68^{d}	61.72^{B}
	2015	63.67^{d}	73.36^{b}	74.29^{ab}	69.41^{c}	69.96^{bc}	77.60^{a}	71.38^{A}

Different lowercase letters in the same row or different uppercase letters in the same column indicate significant differences between treatments.



orch.+ryegr.+alf. – orchard grass + ryegrass + alfalfa (M1); orch.+alf. – orchard grass + alfalfa (M2); ryegr.+alf. – ryegrass + alfalfa (M3); SMS – spent mushroom substrate; CS – cow slurry

Fig. 2.The effect of organic fertilizer combinations (SMS and CS) on hemicellulose content (g·kg^{-1} DM) in legume-grass mixtures across harvests (average values of all growing seasons). Different lowercase letters for each mixture indicate significant differences between treatments. Vertical bars indicate the standard error

Across growing seasons (Table 5), hemicellulose content in forage was similar in the first and second ones, but increased significantly in the third, by about 10 g·kg^{-1}. In the M1 and M2 mixtures, higher hemicellulose content was recorded in subsequent growing seasons because of an increasing dominance of alfalfa in the sward. On the other hand, in the M3 mixture, a much lower hemicellulose content was recorded in the second year. Pappas et al. (2009) found that compared to grasses the content of hemicellulose in alfalfa was three times lower.

Differences between hemicellulose content in different mixtures were statistically significant (Table 6); as an average of all harvests and treatments, it was the highest in alfalfa with orchard grass (72.53 g·kg^{-1}) and the lowest in the mixture of ryegrass with alfalfa (57.06 g·kg^{-1}). However, as a response of mixtures to individual treatments, alfalfa and orchard grass yielded highest of all, with 79.53 g·kg^{-1} (average of three harvests) on the control plot. Ryegrass with alfalfa yielded lowest of all when treated with 10 t of mushroom substrate compost and 60 m^3 of slurry (46.24 g·kg^{-1}). Forage legumes are known to contain less fibre than grasses, and, according to Salama and Nawar (2016), legume-grass forage groups produced significantly higher hemicellulose content than forage legumes.

Hemicellulose content was dependent in a statistically significant way on the harvest (Table 6). As an average for treatments, it significantly increased in subsequent harvests, from 58.39 g·kg^{-1} in the first to 69.54 g·kg^{-1} in the third; it had a similar distribution pattern in the mixtures of orchard grass with alfalfa and ryegrass with alfalfa. However, in their experiment Truba et al. (2017) found that, as an average across treatments and growing seasons, the highest amount of hemicellulose was in the mixture of orchard grass and ryegrass of the first harvest (181.0 and 159.4 g·kg^{-1}, respectively) and the lowest in the third (166.1 and 135.9 g·kg^{-1}). In a similar way, examining hemicellulose content in *Dactylis glomerata,* Ciepiela (2014) found that it was the smallest in the third harvest (220.4 g·kg^{-1} DM).

Table 6.The effect of organic fertilizer combinations on hemicellulose content in legume-grass mixtures across harvests (g·kg^{-1} DM)

Mixture (C)	Harvest (B)	Treatment (A)						Mean
		0	SMS	CS	$SMS_{10} + CS_{60}$	$SMS_{20} + CS_{40}$	$SMS_{30} + CS_{20}$	
M1 – orchard grass + ryegrass + alfalfa	I	45.89	69.51	71.47	46.79	62.83	72.02	61.42
	II	75.10	81.57	81.33	67.58	76.49	49.69	71.96
	III	66.10	59.92	69.72	65.10	68.54	47.98	62.89
M2 – orchard grass + alfalfa	I	78.00	72.63	57.52	69.42	71.47	57.70	67.79
	II	81.78	78.49	55.19	62.48	67.17	80.36	70.91
	III	78.80	80.01	79.85	83.06	65.02	86.44	78.90
M3 – ryegrass + alfalfa	I	44.66	64.31	59.57	26.29	41.78	39.04	45.94
	II	66.50	62.00	62.08	52.91	45.71	61.19	58.40
	III	66.08	80.95	66.35	59.51	60.91	67.24	66.84
Mean effect of harvest								
I		56.18^{d}	68.82^{a}	62.85^{b}	47.50^{e}	58.70^{c}	56.25^{cd}	58.39^{C}
II		74.46^{a}	74.02^{a}	66.20^{b}	60.99^{c}	63.14^{c}	63.74^{bc}	67.09^{B}
III		70.33^{bc}	73.63^{a}	71.98^{b}	69.22^{c}	64.88^{d}	67.22^{cd}	69.54^{A}

Different lowercase letters in the same row or different uppercase letters in the same column indicate significant differences between treatments.

Degree of lignification

According to the data presented in Table 7, the degree of forage lignification was greatly affected by fertilizer treatment. The highest lignin content was in the forage from the control plot (14.32%) (a) and the lowest in plants treated with slurry (13.75%).

Table 7. Dry matter lignification (%) of legume-grass mixtures across treatments and research years

Mixture (C)	Year (B)	Treatment (A)						Mean
		0	SMS	CS	SMS_{10} + CS_{60}	SMS_{20} + CS_{40}	SMS_{30} + CS_{20}	
M1 – orchard grass + ryegrass + alfalfa	2013	15.52	14.14	13.42	15.18	12.93	16.13	14.55
	2014	15.56	14.22	12.71	14.30	14.05	13.99	14.14
	2015	15.77	13.97	13.13	13.23	13.41	12.98	13.75
M2 – orchard grass + alfalfa	2013	13.62	14.16	15.28	13.99	14.32	12.07	13.91
	2014	12.39	12.87	14.19	13.42	12.58	13.12	13.10
	2015	13.18	13.88	14.22	13.76	13.53	13.89	13.74
M3 – ryegrass + alfalfa	2013	13.69	13.89	13.57	14.73	15.76	15.55	14.53
	2014	14.65	14.04	13.92	14.35	14.01	14.87	14.31
	2015	14.46	13.76	13.35	14.23	14.14	13.85	13.97
Average treatment effect								
		14.32^{a}	13.88^{cd}	13.75^{d}	14.13^{b}	13.86^{cd}	14.05^{bc}	14.00
Average growing season effect								
2013		14.28^{b}	14.06^{c}	14.09^{c}	14.63^{a}	14.34^{b}	14.58^{a}	14.33^{A}
2014		14.20^{a}	13.71^{c}	13.60^{c}	14.02^{ab}	13.55^{c}	13.99^{b}	13.85^{B}
2015		14.47^{a}	13.87^{b}	13.57^{c}	13.74^{b}	13.69^{bc}	13.57^{c}	13.82^{B}

Different lowercase letters in the same row or different uppercase letters in the same column indicate significant differences between treatments

Among legume-grass mixtures (Fig. 3), the one with orchard grass and alfalfa (13.58%) had the lowest degree of lignification. In the remaining ones it was significantly higher, with 14.14% for orchard grass with perennial ryegrass and alfalfa and 14.28% for perennial ryegrass with alfalfa. Both of these values were not significantly different. Noteworthy was the fact that, in general, in all legume-grass mixtures (Table 7) the degree of lignification decreased in subsequent research years. The best in this respect was the M2 mixture, with orchard grass and alfalfa (Fig. 3). This may have been due to a change in the botanical composition (Todorov et al. 2010), especially with regard to the proportion of legume plants to grasses. Sanderson (2010) and Erla (2011) reported that NDF content decreased with an increased share of legumes in grass-legume mixtures, while it increased with an increased proportion of grass. Similar results were reported by Lithourgidis et al. (2006), Albayrak et al. (2011), and Albayrak and Turk (2013) for different types of legume-grass mixtures.

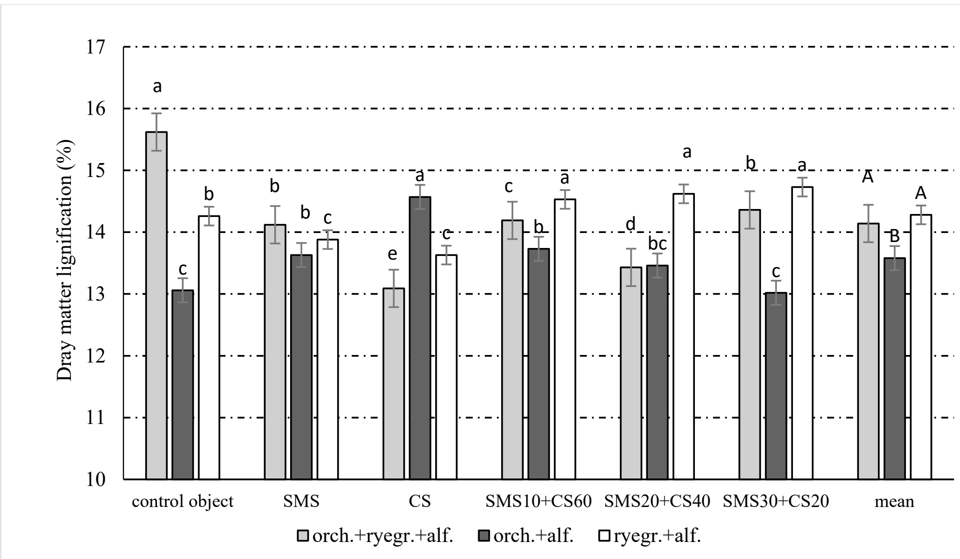
The degree of lignification is an important parameter in the assessment of forage quality (Todorov et al. 2010). It indicates the stage of the lignin formation process in plant cell walls. Lignification changes NDF composition and reduces the digestibility of organic matter. Thus, it lowers the forage energy value and reduces dry matter intake, which, as a consequence, affects the nutritional value of roughage. The process of lignification is greatly affected by fibre fraction content, both ADL and NDF (Salama and Nawar 2016). When NDF content in the forage is higher, the degree of its lignification is lower. In turn, high content of NDF fibre negatively affects the intake of dry matter and energy (Elgersma and Søegaard 2018), which leads to a decrease in the yield of cow's milk and its content of protein and fat.

Assessing the effect of harvests (Table 8) it was observed that the highest degree of lignification was in plants from the first one (14.35%) and the lowest from the second (13.42%). The same relationship was generally confirmed in all legume-grass mixtures.

Table 8. Dry matter lignification (%) of legume-grass mixtures across treatments and harvests

Mixture (C)	Harvest (B)	Treatment (A)						Mean
		0	SMS	CS	SMS$_{10}$ + CS$_{60}$	SMS$_{20}$ + CS$_{40}$	SMS$_{30}$ + CS$_{20}$	
M1 – orchard grass + ryegrass + alfalfa	I	15.26	14.13	13.26	15.19	13.84	13.93	14.27
	II	15.06	13.58	12.53	13.21	12.58	13.70	13.44
	III	16.55	14.66	13.47	14.16	13.88	15.46	14.70
M2 – orchard grass + alfalfa	I	12.87	13.95	15.11	14.68	13.57	13.18	13.89
	II	12.69	13.14	14.77	13.36	13.09	13.04	13.35
	III	13.62	13.79	13.83	13.14	13.72	12.83	13.49
M3 – ryegrass + alfalfa	I	14.75	14.10	13.75	16.27	15.33	15.16	14.89
	II	13.30	12.77	12.82	13.49	14.11	14.39	13.48
	III	14.74	14.76	14.31	13.82	14.41	14.63	14.45
Average harvest effect								
I		14.29^{b}	14.06^{b}	14.04^{b}	15.38^{a}	14.25^{b}	14.09^{b}	14.35^{A}
II		13.68^{a}	13.16^{b}	13.37^{b}	13.35^{b}	13.26^{b}	13.71^{a}	13.42^{B}
III		14.97^{a}	14.40^{b}	13.87^{cd}	13.71^{d}	14.00^{c}	14.31^{b}	14.21^{A}

Different lowercase letters in the same row or different uppercase letters in the same column indicate significant differences between treatments



orch.+ryegr.+alf. – orchard grass + ryegrass + alfalfa (M1); orch.+alf. – orchard grass + alfalfa (M2); ryegr.+alf. – ryegrass + alfalfa (M3); SMS – spent mushroom substrate; CS – cow slurry

Fig. 3. Dry matter lignification (%) of legume-grass mixtures across treatments (SMS and CS) – average for research years. Different lowercase letters for each mixture indicate significant differences between treatments. Vertical bars indicate the standard error

Non-structural carbohydrates

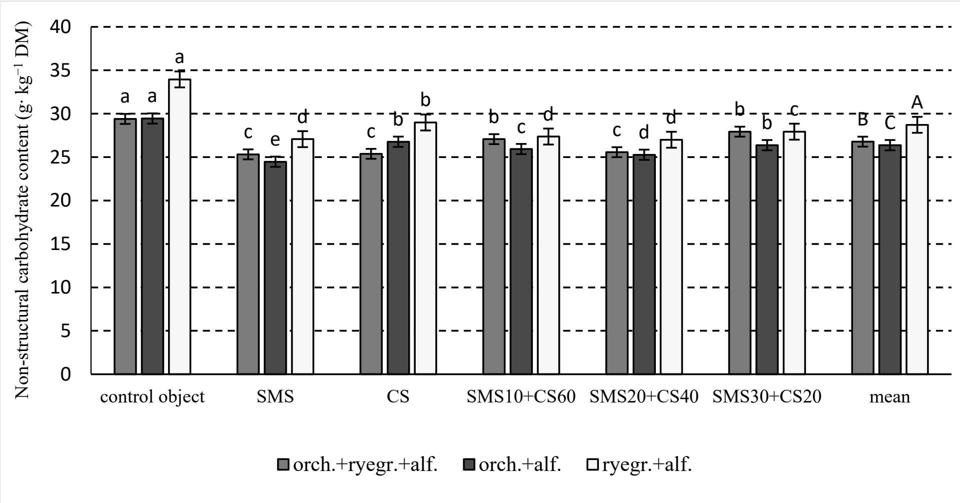
The content of non-structural carbohydrates (Table 9) in forage varied depending on the fertilizer combination. Their greatest amounts were found in the plants from the control plot (300.5 g·kg^{-1}), but organic fertilizer treatment contributed to a decrease in their content in a statistically significant way. Their lowest amount was in plants treated with mushroom substrate compost (257.2 g·kg^{-1}), with Kozłowski and Swędrzyński (2001) and Ciepiela (2014) obtaining similar results for orchard grass. According to Peyraud et al. (2009), carbohydrates are used to produce protein compounds, which is why in the present experiment their content in plants treated with organic fertilizer was low.

Table 9. The effect of organic fertilizer combinations on non-structural carbohydrate content in legume-grass mixtures across growing seasons (g·kg^{-1}DM)

Mixture (C)	Year (B)	Treatment (A)						Mean
		0	SMS	CS	SMS_{10} + CS_{60}	SMS_{20} + CS_{40}	SMS_{30} + CS_{20}	
M1 – orchard grass + ryegrass + alfalfa	2013	297.1	257.9	282.5	263.4	226.8	290.1	269.6
	2014	301.3	248.8	243.4	260.9	257.2	264.4	262.6
	2015	328.2	272.5	287.5	274.0	279.9	282.3	287.4
M2 – orchard grass + alfalfa	2013	286.3	221.3	222.1	266.0	253.2	271.2	253.4
	2014	282.1	241.2	260.9	269.0	237.1	266.7	259.8
	2015	295.6	284.1	283.4	281.4	274.1	280.6	282.8
M3 – ryegrass + alfalfa	2013	292.6	262.0	234.2	252.1	255.4	260.4	259.5
	2014	324.7	265.7	264.3	275.8	261.6	264.8	276.1
	2015	297.0	261.5	272.6	271.9	290.6	270.0	277.3
Mean effect of treatment								
		300.5^{a}	257.2^{e}	261.2^{d}	268.3^{c}	259.5^{e}	272.3^{b}	269.8
Mean effect of year								
2013		292.0^{a}	247.1^{d}	246.3^{d}	260.5^{c}	245.1^{d}	273.9^{b}	260.8^{C}
2014		302.7^{a}	251.9^{d}	256.2^{c}	268.6^{b}	252.0^{d}	265.3^{c}	266.2^{B}
2015		306.9^{a}	272.7^{d}	281.2^{b}	275.8^{c}	281.5^{b}	277.6^{c}	282.5^{A}

Different lowercase letters in the same row or different uppercase letters in the same column indicate significant differences between treatments

There was a considerable variety in the content of non-structural carbohydrates across growing seasons (Table 9). Their greatest amounts with 282.5 g·kg^{-1} were found in 2015, which was significantly higher than in 2013 (260.8 g·kg^{-1}) or in 2014 (266.2 g·kg^{-1}). A similar trend was found in all legume-grass mixtures. On average, the highest content of non-structural carbohydrates was in the M3 mixture. As mentioned above, various combinations of mushroom substrate compost and slurry generally resulted in a significant decrease in non-structural carbohydrate content, but their amounts in plants across individual mixtures varied considerably (Table 10). The greatest amount of non-structural carbohydrates (Fig. 4) was in the mixture of orchard grass with ryegrass and alfalfa (277.1 g·kg^{-1}) and the smallest in orchard grass with alfalfa (265.2 g·kg^{-1}), with the differences being statistically significant. This indicated a better nutritional value and taste of M1 forage.



orch.+ryegr.+alf. – orchard grass + ryegrass + alfalfa (M1); orch.+alf. – orchard grass + alfalfa (M2); ryegr.+alf. – ryegrass + alfalfa (M3); SMS – spent mushroom substrate; CS – cow slurry

Fig. 4.The effect of organic fertilizer combinations (SMS and CS) on non-structural carbohydrate content (g·kg^{-1} DM) in legume-grass mixtures across harvests (average values of all growing seasons). Different lowercase letters for each mixture indicate significant differences between treatments. Vertical bars indicate the standard error

Studies on the content of carbohydrates in meadow plants conducted by Kozłowski and Swędrzyński (2001) and Ciepiela (2014) indicate that orchard grass is a species less resourceful in soluble carbohydrates than perennial or Italian ryegrass.

Table 10. The effect of organic fertilizer combinations on non-structural carbohydrate content in legume-grass mixtures across growing seasons across harvests (g·kg^{-1} DM)

Mixture (C)	Harvest (B)	Treatment (A)						Mean
		0	SMS	CS	SMS_{10} + CS_{60}	SMS_{20} + CS_{40}	SMS_{30} + CS_{20}	
M1 – orchard grass + ryegrass + alfalfa	I	297.4	249.6	253.5	254.0	245.0	267.0	261.1
	II	303.0	246.0	265.5	248.0	255.0	277.0	265.8
	III	316.9	282.0	381.2	292.0	265.0	290.0	304.5
M2 – orchard grass + alfalfa	I	280.0	258.0	262.0	278.0	255.0	280.0	268.8
	II	298.0	253.0	281.5	276.0	244.0	274.0	271.1
	III	286.5	235.0	220.0	263.0	266.0	264.0	255.8
M3 – ryegrass + alfalfa	I	304.6	252.0	246.0	280.2	267.0	291.0	273.5
	II	282.4	235.0	256.0	254.0	259.0	240.5	254.5
	III	415.2	295.0	268.5	266.0	279.0	284.0	301.3
Mean effect of harvest								
I		294.0^{a}	253.2^{d}	253.8^{d}	270.7^{c}	255.7^{d}	279.3^{b}	267.8^{B}
II		294.5^{a}	244.7^{e}	267.7^{b}	259.3^{c}	252.7^{d}	263.8^{b}	263.8^{C}
III		339.5^{a}	270.7^{d}	289.9^{b}	273.7^{d}	270.0^{d}	279.3^{c}	287.2^{A}

Different lowercase letters in the same row or different uppercase letters in the same column indicate significant differences between treatments

Significant differences in the content of non-structural carbohydrates were between harvests (Table 10). Their greatest amount was in the forage from the third harvest (287.2 $g \cdot kg^{-1}$), significantly higher than in the first or second. A clear decrease during the summer can be explained by the increased breathing of plants in high temperature conditions, in which sugars are consumed (Watts 2008). Other authors (Downing and Gamroth 2007) also found that the content of non-structural carbohydrates in grass decreased with an increase in air temperature. Similar seasonal changes in their content in grass were recorded by Kozłowski and Swędrzyński (2001) and Ciepiela (2014).

However, in individual harvests the mixtures varied in non-structural carbohydrate content (Table 10). In the third one it was the highest in the mixtures of orchard grass with ryegrass and alfalfa and in ryegrass with alfalfa (304.5 and 301.3 $g \cdot kg^{-1}$, respectively) and in the second harvest in the mixture of orchard grass with alfalfa (271.1 $g \cdot kg^{-1}$).

CONCLUSION

The most cellulose, the least hemicelluloses, and the highest degree of lignification were recorded in the mixture of ryegrass with alfalfa, while the degree of lignification was the smallest in the forage of alfalfa and orchard grass, which also contained the least cellulose but the most hemicellulose. On average, the highest amount of cellulose was in the biomass of the first harvest and the least in the third. Contrary to that, the least amount of hemicellulose was in the first harvest and the most in the third. Compared to slurry, mushroom substrate compost considerably increased the content of both cellulose and hemicellulose in plants. The content of non-structural carbohydrates was dependent on fertilizer treatment. The treatments reduced the content of non-structural carbohydrates, compared to control, with 30 t mushroom substrate compost and 20 m^3 of slurry being the most effective. Their greatest amount was in the forage from the control plot and in the mixture of orchard grass with perennial ryegrass and alfalfa, and the lowest in the one with orchard grass and alfalfa.

From a practical point of view, the cultivation of orchard grass with alfalfa treated with mushroom substrate compost should be recommended because of its lower cellulose content and lower degree of lignification. However, considering the lower content of hemicellulose and the higher content of non-structural carbohydrates, the cultivation of ryegrass with alfalfa treated with mushroom substrate compost and liquid manure would be also advisable. All the above findings confirmed the hypotheses formed at the beginning of the experiment. Further research is needed to investigate the effects of the combined application of mushroom substrate compost and slurry on other forage crops.

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ZMIANY WĘGLOWODANÓW W MIESZANKACH MOTYLKOWO-TRAWIASTYCH POD WPŁYWEM PODŁOŻA POPIECZARKOWEGO I GNOJOWICY BYDLĘCEJ

Streszczenie. Celem badań była ocena wpływu podłoża popieczarkowego i gnojowicy na zawartość węglowodanów strukturalnych i niestrukturalnych w mieszance lucerny z trawami. Trzyletnie badania przeprowadzono w latach 2013–2015. W doświadczeniu zastosowano dwa czynniki badawcze: (1) podłoże po produkcji pieczarki białej, gnojowicę bydlęcą, w różnych kombinacjach; (2) trzy mieszanki traw z lucerną: kupkówkę pospolitą, życicę trwałą, lucernę mieszańcową (M1); kupkówkę pospolitą, lucernę mieszańcową (M2); życicę trwałą, lucernę mieszańcową (M3). W każdym sezonie wegetacyjnym mieszanki zbierano trzykrotnie. W materiale roślinnym określono zawartość suchej masy, włókna neutralno-detergentowego (NDF), włókna kwaśno-detergentowego (ADF), ligniny kwaśno-detergentowej (ADL), celulozy, hemicelulozy, ligniny, białka ogólnego, popiołu surowego i tłuszczu surowego za pomocą spektroskopii w bliskiej podczerwieni (NIRS), przy użyciu spektrometru NIRFlex N-500. Podłoże popieczarkowe spowodowało zwiększenie zawartości celulozy i hemicelulozy w mieszankach motylkowo-trawiastych w porównaniu z gnojowicą bydlęcą. Najwięcej celulozy, najmniej hemicelulozy i najwyższy stopień lignifikacji odnotowano w mieszance życicy z lucerną. Natomiast w mieszance lucerny z kupkówką pospolitą stwierdzono znacznie mniejszy stopień lignifikacji, najmniej celulozy, ale najwięcej hemicelulozy. Średnio najwięcej celulozy odnotowano w biomasie pierwszego zbioru, a najmniej – w biomasie trzeciego.

Słowa kluczowe: celuloza, hemiceluloza, stopień lignifikacji, węglowodany niestrukturalne.