Fiber Fraction Content in Legume–Grass Mixtures Treated with Mushroom Substrate and Cow Slurry

Kazimierz Jankowski and Elżbieta Malinowska*

ABSTRACT
The work reported here was undertaken to determine the effect of spent mushroom substrate (SMS) and cow slurry used in the cultivation of hybrid alfalfa and grass mixtures on plant productivity and dry matter nutritional value. Uncomposted waste left after growing mushrooms (Agaricus bisporus) was used. Additionally, slurry from cows with the milk yield of 6000 to 8000 litters a year was applied; the animals were housed indoors on straw bedding, with manure removed daily. The 3-yr research study (2013–2015) was conducted in the experimental field, in east-central Poland. Treatments were replicated three times, with plots of 3 m³ as experimental units in a split-plot arrangement. In the experiment the main research factors were legume–grass mixtures and organic fertilizers with high organic matter content: SMS and cow slurry, used separately and in various combinations. In the experiment three plant species were involved: Medicago sativa x varia Martyn (alfalfa hybrid) ‘Tula’, Dactylis glomerata (orchard grass) ‘Bora’, and Lolium perenne L. (perennial ryegrass) ‘Info’. These species were grown as three legume–grass mixtures. The results demonstrated that SMS application to legume–grass mixtures increased NDF content in the forage more than slurry. The content of the ADF and ADL fractions and the yields of legume–grass mixtures were higher in the forage from plots with slurry than from plots with SMS. The mixture of orchard grass with alfalfa produced forage of the best quality with regard to the content of fiber fractions.

Core Ideas
- Mushroom substrate application to legume mixtures increased neutral detergent fiber content in the forage more than slurry.
- The mixture of orchard grass with alfalfa produced forage of the best quality, with regard to the content of fibre fractions.
- The content of the acid detergent fiber and acid detergent lignin fractions and yielding of legume grass mixtures were higher in the forage from plots with slurry than from plots with mushroom.

IN THE production of animal feed not only the yield is important but also its quality. Well balanced forage from legume–grass mixtures meets these requirements. According to Gawel (2008a) such mixtures play a large role both in integrated and in organic production systems. They are a source of very valuable feed of favorable chemical composition, containing components necessary in animal nutrition (Kryszak, 2003; Gawel, 2008b). In addition, many authors (Aufrere et al., 2008) point out that forage mixtures leave the soil in good condition for subsequent plants and have a positive effect on its nutrient content (Kryszak, 2003). In organic farming adequate fertilization, not harmful to the environment, is important, but, at the same time, it should positively affect the yield and quality of crops. For all those purposes manure and SMS can be used.

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 SMS. 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, SMS is exceptionally valuable organic fertilizer that can be used in agriculture. Research has been performed into the use of SMS on arable land (Guo and Chorover, 2004; Hackett, 2015), on grassland (Rak et al., 2001; Jankowski et al., 2005; Ciepiela et al., 2007; Wiśniewska-Kadżajan, 2013a, 2013b; Malinowska et al., 2017), in market gardening (Kuśmirek et al., 2012), in horticulture (Uzun, 2004; Danai et al., 2012), and on lawns (Wiśniewska-Kadżajan, 2013c, 2013d). The application of SMS has a beneficial effect on an increase in the crop yield (Wiśniewska-Kadżajan, 2013a; Kuśmirek et al., 2012; Wiśniewska-Kadżajan and Jankowski, 2015). Additionally, a similar increase in protein content of Dactylis glomerata has also been demonstrated (Wiśniewska-Kadżajan and Jankowski, 2015). Taking into account animals’ nutrient requirements Wiśniewska-Kadżajan (2013c, 2013d) found that in forage fertilized with SMS there is optimum content of potassium and magnesium, with deficiency of calcium and sodium.

Another valuable fertilizer is cow slurry. Staszewski and Biś (2011) reported that its nutrients are in forms more easily available to plants than, for example, those present in manure. Slurry contains quick-releasing nutrients, and, due to its high quantities of microorganisms, it can increase availability of nutrients in the soil and those present in other waste materials. The combination of these two types of organic fertilizer, i.e., manure and SMS, can
contribute to improving the yield of crop plants and their nutritional values (Malinowska et al., 2017). Hybrid alfalfa, one of the most nutritious plants of the legume family, can be cut three to four times a year and can be used fresh, as hay, or silage (Gawel, 2008a). In the experiment it was grown as a mixture with grass.

The aim of the study was to evaluate the effect of SMS and cow slurry used in the cultivation of hybrid alfalfa and grass mixtures on plant productivity and dry matter nutritional value.

**MATERIALS AND METHODS**

Set up in the autumn of 2012 and lasting from 2012 to 2015, the 3-yr research study was conducted in the experimental field of the Department of Grasslands and Landscape Architecture Development, the University of Humanities and Natural Sciences in Siedlce, in east-central Poland. Each treatment was replicated three times, with plots of 3 m³ as experimental units in a split-plot arrangement. There were three experimental factors in the study: (i) treatment, with SMS and cow slurry used separately and in various combinations; (ii) legume–grass mixtures; and (iii) harvests.

The experiment consisted of the following units:

1. control unit (no fertilization) (0)
2. spent mushroom substrate (30 t ha⁻¹) (SMS)
3. cow slurry (60 m³ ha⁻¹) (CS)
4. mushroom substrate (10 t ha⁻¹) + cow slurry (60 m³ ha⁻¹) (SMS₁₀ + CS₆₀)
5. mushroom substrate (20 t ha⁻¹) + cow slurry (40 m³ ha⁻¹) (SMS₂₀ + CS₄₀)
6. mushroom substrate (30 t ha⁻¹) + cow slurry (20 m³ ha⁻¹) (SMS₃₀ + CS₂₀)

In the experiment three plant species were involved: *Medicago sativa* x *varia* Martyn (alfalfa hybrid) of the variety ‘Tula’, *Dactylis glomerata* (orchard grass) of variety ‘Bora’, and *Lolium perenne* (perennial ryegrass) of durable variety ‘Info’. Taking into account differences in germination capacity, the sowing rate of plants grown on their own was as follows: hybrid alfalfa at 23 kg ha⁻¹, *D. glomerata* at 21 kg ha⁻¹, and *L. perenne* at 31 kg ha⁻¹. These species were grown as three legume and grass mixtures: M1, a mixture of *D. glomerata*, *L. perenne*, and *M. sativa* x *varia*; M2, a mixture of *D. glomerata* and *M. sativa* x *varia*; and M3, a mixture of *L. perenne* and *M. sativa* x *varia*. In these mixtures, the share of each component was the same.

In the experiment, organic fertilizer was used in the form of cow slurry and SMS. The SMS was applied once at the start of the experiment, before the growing season, in different doses mixed with the soil. Cow slurry was used every year throughout the experiment in equal doses after each harvest, according to the experimental design. Table 1 presents chemical composition of cow slurry and SMS. The latter was taken from a mushroom farm immediately after the end of a production cycle lasting 60 to 64 d. Slurry was collected from cows producing 6000 to 8000 L of milk a year and housed indoors on straw bedding, with manure removed daily. Before seeds were planted, SMS was mixed with a 20- to 25-cm layer of the soil. Slurry was applied before each growing cycle with control plots treated with the same amount of water. On plots with lower amounts of slurry additional quantities of water were used so that the amount of liquids was the same on each unit.

The experiment was set up on the soil of the granulometric composition of loamy sand, of the anthropogenic order, the type of culture earth soil, and the subtype of hortisole. Chemical analysis of the soil showed high content of absorbable forms of phosphorus and magnesium. However, absorbable forms of potassium were within the limits of the average content. Carbon content in soil organic compounds \(C_{org}\) was 13.50 g kg⁻¹ of dry matter (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.

Sielianinov’s hydrothermal coefficient \(K\) was calculated to determine temporal variation of meteorological elements and their effects on vegetation. Sielianinov’s coefficient was calculated with the following formula (Skowera and Pula, 2004):

\[
K = \frac{P \times 10}{\sum t}
\]

where \(P\) is monthly rainfall and \(\sum t\) is the sum of mean day temperatures.

During each growing season all the mixtures were harvested three times, with the first harvest at the end of May, the second at the beginning of July, and the third in mid-September. Fresh matter from each plot was weighed with the AG 199 hook scales (up to 40 kg) and a sample of 0.6 kg was collected. Using the SLN 32 laboratory dryer of the POL-EKO-APARATURA company the plant material was dried at 105°C until it reached a stable weight. Then the samples were ground with the Wż-1S laboratory grinder made by the Research Institute of the Bakery Industry in Bydgoszcz. The plant material was used to determine dry matter content and to perform chemical analysis to measure the amount of crude fiber fractions of NDF, ADF, and ADL. The chemical composition of the plants was determined with near-infrared spectroscopy \(\text{(NIRS)}\) using the NIRFlex N-500 spectrometer. Chemical analysis of the plant material was performed by the Institute of Technology and Life Sciences in Falenty with the staff having relevant experience and knowledge to operate the equipment. The results of the research were processed statistically using three-factor analysis of variance. The Fisher-Snedecor test was done to determine the significance of the effects of experimental factors on the parameters tested in the research. Tukey’s test was used to compare means at the LSD 0.05 significance level. All the calculations were made with Statistica 10.0 (StatSoft, Inc., 2011).

**RESULTS**

**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 periods weather conditions were not as favorable, varying from extremely dry in August 2015 to

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Spent mushroom substrate</th>
<th>Cow slurry</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (g kg⁻¹ DM)</td>
<td>24.50</td>
<td>48.00</td>
</tr>
<tr>
<td>P (g kg⁻¹ DM)</td>
<td>9.50</td>
<td>12.64</td>
</tr>
<tr>
<td>K (g kg⁻¹ DM)</td>
<td>13.20</td>
<td>43.16</td>
</tr>
<tr>
<td>Ca (g kg⁻¹ DM)</td>
<td>58.20</td>
<td>30.75</td>
</tr>
<tr>
<td>Dry matter content (%)</td>
<td>25</td>
<td>10</td>
</tr>
</tbody>
</table>
extremely wet in May 2013. Throughout the experiment the best conditions were at the beginning of each growing period. It can be concluded that the most difficult situation for plants was in 2015, when, apart from May and the end of the growing period, the weather ranged from moderately dry to extremely dry.

**Dry Matter Yield**

The forage yield was dependent on the type of fertilizer and the type of mixture (Fig. 1). The effect of different treatments on the yield of dry matter, as the 3-yr average, was the highest as a result of the application of slurry on its own (8.20 t ha⁻¹), and when 10 t of SMS was used together with 60 m³ of slurry (8.09 t ha⁻¹). The lowest yield of dry matter was from the control (4.60 t ha⁻¹). Taking into account fertilized units only, the lowest amount of dry matter was produced on the plot with 30 t of SMS applied together with 20 m³ of liquid manure (7.65 t ha⁻¹).

Studying the effect of different legume–grass mixtures and fertilizer combinations (Fig. 1) it was found that all three of the former reacted to organic fertilization with a statistically significant increase in the dry matter yield. Orchard grass with ryegrass and alfalfa and the mixture of ryegrass with alfalfa had the highest yield of 8.39 and 8.18 t ha⁻¹, respectively, on the unit treated with cow slurry on its own. In the case of orchard grass with alfalfa, the highest yield (8.68 t ha⁻¹) was on the plot with 10 t of SMS and 60 m³ of cow slurry.

**Neutral-Detergent Fiber Content**

Neutral-detergent fiber content was generally varied depending on the treatment, the type of mixture, and the harvest (Table 3). With regard to the fertilizer effect (Table 3), NDF content was the highest in the forage from the unit with 30 t of SMS and 20 m³ of liquid manure (393.93 g kg⁻¹). The lowest content of this parameter was in the forage from the control (375.95 g kg⁻¹). Based on research results it can be concluded that a higher dose of substrate in a statistically significant way increased the content of NDF in the mixtures, compared with unfertilized control.

Only the mixture of ryegrass and alfalfa and that with orchard grass, ryegrass and alfalfa reacted with an increased NDF content after SMS application (Fig. 2). In the mixture of orchard grass with alfalfa it increased only when it was treated with SMS together with slurry. The highest amount of NDF was found in mixture M2 (orchard grass + alfalfa), when treated with 30 t of SMS and 20 m³ of liquid manure (398.33 g kg⁻¹). It was the lowest in the forage of ryegrass with alfalfa (364.10 g kg⁻¹) after application of 10 t of SMS with 60 m³ of slurry. On plots with orchard grass + ryegrass + alfalfa or ryegrass + alfalfa, slurry was less effective than SMS in terms of increasing NDF content. However, the mixtures reacted with higher NDF content when a proportion of slurry in cow slurry and SMS combined application was lower. On plots with the orchard grass and alfalfa mixture, neither slurry nor substrate applied only were very effective in increasing it, but when they were applied together the NDF content compared with control increased.

There were statistically significant differences in average NDF content between grass mixtures. It was dependent on the treatment (Fig. 2) and the harvest. Orchard grass and alfalfa had the highest amount of neutral detergent fiber (390.06 g kg⁻¹), while ryegrass with alfalfa the lowest (378.74 g kg⁻¹). With regard to the cuts (Table 3), forage from the second harvest had the highest average NDF content (387.91g kg⁻¹), but it was the lowest in the third one (380.08 g kg⁻¹). It was found (Table 3) that in the mixture of ryegrass with alfalfa NDF content increased between the first harvest and the second harvest and then it decreased between the second and third harvests. The highest value was recorded in the mixture of orchard grass with alfalfa in the first harvest (392.88 g kg⁻¹), while in the mixture of perennial ryegrass with alfalfa the highest statistically significant average content was in the second harvest and the lowest in the second one (373.88 g kg⁻¹).

**Acid Detergent Fiber Content**

Content of acid detergent fiber (ADF) in dry matter was dependent on the applied fertilizer, the mixture, and the harvest (Table 4). When considering different treatments, the largest ADF content, average for all mixtures and harvests, was in the plants treated with 30 t of SMS and 20 m³ of liquid manure (331.52 g kg⁻¹). The smallest content of ADF was on the plot without fertilizer (308.96 g kg⁻¹). On plots where SMS was applied the forage, in a statistically significant way, contained more acid detergent fiber than on units with slurry. It can be

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Table 2. Value of Sielianinov’s hydrothermal coefficient (K) in the growing seasons, calculated from monthly rainfall and the sum of mean day temperatures.

<table>
<thead>
<tr>
<th>Year</th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>2.56</td>
<td>3.07</td>
<td>2.11</td>
<td>0.84</td>
<td>0.78</td>
<td>2.53</td>
<td>0.60</td>
</tr>
<tr>
<td>2014</td>
<td>1.36</td>
<td>1.87</td>
<td>1.64</td>
<td>0.59</td>
<td>1.92</td>
<td>0.64</td>
<td>0.12</td>
</tr>
<tr>
<td>2015</td>
<td>1.22</td>
<td>2.63</td>
<td>0.87</td>
<td>1.08</td>
<td>0.18</td>
<td>1.46</td>
<td>1.94</td>
</tr>
</tbody>
</table>

† Values are rated as: 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); and K > 3.0, extremely wet (ew).
concluded that SMS used as fertilizer makes plants stronger, with higher amount of indigestible components of cell walls. ADF content in the forage (Table 4), as an average for all treatments and mixtures, was the highest in the first harvest (327.39 g kg⁻¹), with the lowest in the third (310.54 g kg⁻¹). In general, the content of this fraction, as an average for all treatments, declined significantly in the forage of subsequent harvests.

Of all mixtures (Fig. 3) the highest fiber content, statistically significant, was in the one with ryegrass and alfalfa (321.88 g kg⁻¹). The statistically significant lowest content of ADF was in orchard grass with alfalfa (317.53 g kg⁻¹).

The effect of fertilizer treatment on the content of acid detergent fiber (Fig. 3) was the highest when 30 tons of SMS and 20 m³ of slurry were applied to the M1 mixture of orchard grass, ryegrass and alfalfa (336.01 g kg⁻¹), and when the same doses were used on the M2 mixture of ryegrass and alfalfa (335.06 g kg⁻¹). On the plots with 20 tons of SMS and 40 m³ of cow slurry, the mixture of orchard grass and alfalfa had the highest yield of 328.43 g kg⁻¹. In all three mixtures the smallest ADF content was recorded on plots without fertilization: 306.35, 304.80 and 315.73 g kg⁻¹, respectively.

Acid Detergent Lignin Content

The content of acid detergent lignin (ADL) fluctuated and was dependent on the type of fertilizer, the mixture, and the harvest (Table 5). Regarding the effect of fertilizer treatment, average for all the mixtures, forage treated with 30 t of SMS and 20 m³ of cow slurry had the greatest acid lignin content (55.23 g kg⁻¹), while the smallest (52.55 g kg⁻¹) was on the plot with slurry. Of all mixtures (Fig. 4) orchard grass with ryegrass and alfalfa had the highest content of acid detergent lignin (54.31 g kg⁻¹).

Table 3. Effect of organic fertilizers spent mushroom substrate (SMS) and cow slurry (CS) on neutral detergent fiber (NDF) content (g kg⁻¹ DM) in legume–grass mixtures (average for the 3 yr).†

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Harvest</th>
<th>Treatment</th>
<th>Mean effect of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orchard grass + ryegrass + alfalfa</td>
<td>I</td>
<td>369.89 SMS 402.41 CS 388.95 SMS10 + CS 377.51 SMS20 + CS 40 SMS30 + CS 383.91 Mean 420.71 390.56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>372.34 SMS 414.81 CS 393.70 SMS10 + CS 383.03 SMS20 + CS 40 SMS30 + CS 382.10 Mean 390.31 389.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>363.91 SMS 375.61 CS 382.85 SMS10 + CS 373.68 SMS20 + CS 40 SMS30 + CS 383.46 Mean 366.69 374.37</td>
<td></td>
</tr>
<tr>
<td>Orchard grass + alfalfa</td>
<td>I</td>
<td>384.11 SMS 378.73 CS 378.78 SMS10 + CS 399.56 SMS20 + CS 40 SMS30 + CS 401.37 Mean 414.73 392.88</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>386.22 SMS 393.54 CS 363.63 SMS10 + CS 390.78 SMS20 + CS 40 SMS30 + CS 402.51 Mean 404.21 390.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>382.65 SMS 379.88 CS 396.67 SMS10 + CS 402.38 SMS20 + CS 40 SMS30 + CS 385.24 Mean 376.06 387.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>368.22 SMS 411.21 CS 377.84 SMS10 + CS 395.26 SMS20 + CS 40 SMS30 + CS 356.02 Mean 393.82 373.88</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>379.66 SMS 385.17 CS 392.41 SMS10 + CS 395.26 SMS20 + CS 40 SMS30 + CS 356.16 Mean 396.62 384.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>376.54 SMS 385.17 CS 382.76 SMS10 + CS 379.87 SMS20 + CS 40 SMS30 + CS 379.80 Mean 382.22 378.74</td>
<td></td>
</tr>
</tbody>
</table>

† For A is treatment; B is mixture, and C is harvest, LSDₐ₀.₀₅: A = 6.22; B = 3.60; C = 3.60; B/C = 6.24; C/B = 6.24; A/B = 10.77; A/C = 7.62; B/A = 8.83.

Table 4. Effect of organic fertilizers spent mushroom substrate (SMS) and cow slurry (CS) on acid detergent fiber (ADF) content (g kg⁻¹ DM) in legume–grass mixtures (average for the 3 yr).†

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Harvest</th>
<th>Treatment</th>
<th>Mean effect of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orchard grass + ryegrass + alfalfa</td>
<td>I</td>
<td>324.00 SMS 332.90 CS 317.48 SMS10 + CS 330.72 SMS20 + CS 40 SMS30 + CS 321.08 Mean 348.69 329.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>297.24 SMS 333.24 CS 312.37 SMS10 + CS 315.45 SMS20 + CS 40 SMS30 + CS 305.61 Mean 340.62 317.42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>297.81 SMS 315.69 CS 313.13 SMS10 + CS 308.58 SMS20 + CS 40 SMS30 + CS 314.92 Mean 318.71 311.47</td>
<td></td>
</tr>
<tr>
<td>Orchard grass + alfalfa</td>
<td>I</td>
<td>306.11 SMS 306.10 CS 321.26 SMS10 + CS 330.14 SMS20 + CS 40 SMS30 + CS 329.90 Mean 357.03 325.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>304.44 SMS 315.05 CS 308.44 SMS10 + CS 328.30 SMS20 + CS 40 SMS30 + CS 335.34 Mean 323.85 319.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>303.85 SMS 299.87 CS 316.82 SMS10 + CS 319.32 SMS20 + CS 40 SMS30 + CS 320.04 Mean 289.62 308.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>323.56 SMS 346.90 CS 318.27 SMS10 + CS 299.89 SMS20 + CS 40 SMS30 + CS 324.24 Mean 354.78 327.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>313.16 SMS 323.17 CS 330.33 SMS10 + CS 342.35 SMS20 + CS 40 SMS30 + CS 310.41 Mean 335.43 325.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>310.46 SMS 316.31 CS 299.38 SMS10 + CS 311.36 SMS20 + CS 40 SMS30 + CS 318.89 Mean 314.98 311.90</td>
<td></td>
</tr>
</tbody>
</table>

† For A is treatment; B is mixture, and C is harvest, LSDₐ₀.₀₅: A = 3.20; B = 1.85; C = 1.85; B/C = 3.21; C/B = 3.21; A/B = 5.54; A/C = 3.92; B/A = 4.54.
Similar content (54.01 g kg\(^{-1}\)) was reported also in the mixture of ryegrass and alfalfa. Orchard grass with alfalfa contained the statistically significant lowest amount of lignin (52.93 g kg\(^{-1}\)). The mixture of orchard grass with alfalfa, contrary to the other two mixtures, reacted to slurry application with a statistically significant increase in ADL content. The use of SMS, both in the first (M1) and third (M3) mixtures led to an increase of this content. Thus, it was found that the level of lignin was dependent on different species, and that the mixtures reacted in different ways to the applied fertilization.

The highest concentration of the ADL fraction (55.25 g kg\(^{-1}\)), as an average for all mixtures and treatments, was noted in the forage from the first harvest, and the lowest (52.04 g kg\(^{-1}\)) from the second harvest (Table 5). The ADL concentration (Table 5) in the individual mixtures in the first harvest was as follows: 55.66 g kg\(^{-1}\) in M1, 54.55 g kg\(^{-1}\) in M2, and 55.53 g kg\(^{-1}\) in M3. In the second harvest the concentration was lower: 52.32, 52.01, and 51.78 g kg\(^{-1}\), respectively. The differences in acid lignin content in the plants were statistically significant.

### DISCUSSION

The results showed that the yield of dry matter was dependent on the type of fertilization and on the mixture. Generally, the yield from plots with fertilizer treatments was significantly higher than in the control. It was confirmed by Wiśniewska-Kadżajan and Jankowski (2015), who found that natural fertilizer increased the yield of grassland forage.

The research also indicated that regardless of the applied fertilizer the yield of all three mixtures was at a similar level and the differences were not statistically significant. It might have been caused by a high proportion of alfalfa in the mixture. Sosnowski and Jankowski (2010) reported slightly higher yields of alfalfa with grass, but weather conditions which prevailed during the growing season were much more favorable compared to those during the present research. Jodełka et al. (2006) pointed out that the yield of legume–grass mixtures was to a much extent dependent on natural factors, such as the air temperature and the amount and distribution of rainfall. According to Chmura et al. (2009) less rain than the optimum can cause a decrease in the alfalfa yield from 5% to up to 23%. In the

### Table 5. Effect of organic fertilizers spent mushroom substrate (SMS) and cow slurry (CS) on acid detergent lignin (ADL) content (g kg\(^{-1}\) DM) in legume–grass mixtures (average for the 3 yr).†

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Harvest</th>
<th>Treatment</th>
<th>Mean effect of treatment</th>
<th>Mean effect of harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>SMS</td>
<td>CS</td>
</tr>
<tr>
<td>Orchard grass + ryegrass + alfalfa</td>
<td>I</td>
<td>56.44</td>
<td>56.85</td>
<td>51.57</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>56.09</td>
<td>56.33</td>
<td>49.35</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>60.21</td>
<td>55.07</td>
<td>51.57</td>
</tr>
<tr>
<td>Orchard grass + alfalfa</td>
<td>I</td>
<td>49.44</td>
<td>52.85</td>
<td>57.25</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>49.00</td>
<td>51.72</td>
<td>53.70</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>52.11</td>
<td>52.40</td>
<td>54.86</td>
</tr>
<tr>
<td>Ryegrass + alfalfa</td>
<td>I</td>
<td>54.33</td>
<td>57.99</td>
<td>51.96</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>50.50</td>
<td>49.20</td>
<td>50.31</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>55.42</td>
<td>58.62</td>
<td>52.34</td>
</tr>
</tbody>
</table>

† For A is treatment; B is mixture, and C is harvest, LSD\(_{0.05}\): A = 0.722; B = 0.418; C = 0.418; B/C = 0.725; C/B = 0.725; C/A = 0.725; A/B = 1.251; A/C = 0.885; B/A = 1.03.
ruminants. It helps maintaining forage structure and provides physical ballast filling the rumen (Andrzejewska et al., 2017). It is also a source of energy for rumen microorganisms. Alfalfa protein breaks down very quickly, and because of that, if there is not enough energy for its assimilation, it is not digested and is lost, leading to a decrease in the milk yield and to lower content of its protein (Fustini et al., 2017). In addition, the knowledge of NDF content allows calculating forage intake necessary for animals (Aufrere et al., 2008).

In the modern system of animal nutrition NDF and ADF (Fustini et al., 2017) are extensively used instead of crude fiber (Jonavičienė et al., 2008; Salama et al., 2008; Doepel et al., 2012; Stejskalova et al., 2013). Desirable content of this parameter in hay for dairy cattle is approximately 400 g kg⁻¹ (Righi et al., 2017). However, in the present experiment the content of NDF in legume–grass forage was a bit lower, with approximately 380 g kg⁻¹. It was also lower than the data obtained by Grzelak and Bocian (2009), who in the forage of permanent meadows recorded from 412 to 501 g kg⁻¹ DM of the NDF fraction. Tomic et al. (2012) demonstrated a much larger content of NDF in *D. glomerata* and *L. perenne*, with 636 and 527 g kg⁻¹ DM, respectively. Additionally, Jankowska-Huflejt and Wróbel (2008) recorded much higher content of the NDF fraction in plants of organic meadows and pastures, ranging from 514 to 616 g kg⁻¹ DM.

The results showed that the application of SMS statistically increased the content of neutral-detergent fiber in alfalfa–grass mixtures. The opposite effect of organic fertilizer application to meadows was described by Wróbel et al. (2013), with manure and slurry treatment resulting in a significant reduction of this parameter.

There were statistically significant differences between legume–grass mixtures in NDF fraction content as an average for all treatments and mixtures. The highest content of neutral fiber was in orchard grass with alfalfa (390.06 g kg⁻¹), while the smallest in ryegrass with alfalfa (378.94 g kg⁻¹).

In the studies of Sosnowski (2012a; Tomic et al., 2012) recorded higher amounts of the NDF fraction in *D. glomerata* than in *L. perenne*. In the present study it was found that plants of the second harvest had the highest NDF content, average for all treatments and mixtures, and its amount in the third was the lowest.

Similar results were reported by Sosnowski and Jankowski (2013), who in the mixture of *Festulolium* with alfalfa also found the largest concentration of this parameter in the second harvest. Andrzejewska et al. (2017) found in their studies that NDF content in individual harvests of alfalfa was closely related to the development stage in which the plants were harvested. If the forage was harvested in the budding or flowering stages, the content of this parameter decreased in the next harvest. Authors believed that during the first growing cycle plants were taller and therefore contained more neutral fiber.

The ADF fraction is the least digestible, containing such forage components as cellulose, lignin, and silica (Brzóska and Śliwiński, 2011). Salama et al. (2008) and Brogna et al. (2018) point out that the more of the ADF is in the ration, the worse is its digestibility. According to Godlewksa and Ciepiela (2016) the optimal acid detergent fiber content in the forage is from 190 to 210 g kg⁻¹.

In the present study the content of ADF was higher and ranged from 308.96 to 331.52 g kg⁻¹. In similar studies Grzelak and Bocian (2009) reported a similar content of the ADF fraction in hay (277–343 g kg⁻¹ DM), while Jankowska-Huflejt and Wróbel (2008) found amounts close to it in pasture green matter (285–300 g kg⁻¹ DM). In turn, Sosnowski (2012a) obtained a higher content of the ADF fraction in *L. perenne* and *D. glomerata*, amounting to 334 and 356 g kg⁻¹ DM, respectively. Much higher content of ADF in ADL of the forage of the same two species was also recorded by Tomic et al. (2012), amounting for *D. glomerata* to 374 g kg⁻¹, and for *L. perenne* to 326 g kg⁻¹.

In the present study the forage from the plots where SMS was used contained much more ADF than from the units with cow slurry. It might have been caused by the fact that slurry contained more nitrogen, and it was absorbed by plants in higher quantities increasing protein content and decreasing the amount of fiber in plants. It turned out that the largest ADF fraction content, as an average for treatments and mixture, was in the third year of studies, in which a drought lasted for most months. According to Chmura et al. (2009) high temperatures and reduced humidity had a large impact on changes in the chemistry of plant cell walls and on deterioration in forage quality.

Alfalfa with ryegrass had statistically the highest content of acid detergent fiber (321.88 g kg⁻¹), taken as an average for all treatments and harvests. Similar acid detergent fiber content (ADF) was recorded in a study of Sosnowski (2012b), who in a mixture of *Festulolium* with alfalfa recorded the level of this fiber fraction at 310 g kg⁻¹. Meadow hay studied by Grzelak and Bocian (2009) contained the same amounts. Tomic et al. (2012) reported statistically significant higher content of the ADF fraction in orchard grass (374.4 g kg⁻¹) than in perennial ryegrass (326 g kg⁻¹). These results were completely different to those obtained in the present experiment, but here the content of this fraction was affected by the fact that alfalfa was a component of the mixture. This plant according to Gawel (2008a), depending on the variety, contains from 418 to 437 g kg⁻¹ of ADF.
The ADF fiber fraction content, as an average for all treatments and mixtures, was the highest in the first harvest in each growing season (327.39 g kg⁻¹), while it was the lowest in the third (310.54 g kg⁻¹). The content of this fraction decreased in a statistically significant way in the forage of subsequent harvests. A decrease of acid detergent fiber content in subsequent alfalfa harvests was also confirmed by the study of Andrzejewska et al. (2017). The authors demonstrated that the amount of this fraction was closely related to the height of plants. Sosnowski and Jankowski (2013) found very similar results, studying the content of the ADF fraction in the mixture of alfalfa with Festulolium, with 306.8 g kg⁻¹ in the first harvest and 322.8 g kg⁻¹ in the third. However, in their experiment acid detergent fiber content slightly increased in the forage of subsequent harvests. In that case, in the years in which the experiment was performed the hydrothermal coefficient was very high and no summer drought was recorded, indicating good conditions for the development of plants.

The content of ADL is an indicator of how advanced plant lignification process is (Kotlarz et al., 2010). Lignin makes it difficult for bacteria to get attached to cell walls, which results in limited digestion. As the ADL content in feed increases, its digestibility worsens (Kotlarz et al., 2010).

By analyzing research results it can be concluded that SMS increased the content of the ADL fraction, while for slurry the relation is opposite. Reduction of acid fiber content in forage after slurry application was also demonstrated by Wróbel et al. (2013). The content of ADL in the forage ranged in the present experiment from 48.08 to 60.21 g kg⁻¹. Wróbel et al. (2013) recorded much less of this fraction, about 45 g kg⁻¹, in the dry matter of forage from permanent meadows.

The area of east-central Poland and especially the Masovian Voivodeship, dominates in the cultivation of mushrooms. Land-use structure in this administrative unit is composed mainly of arable land and pastures, with a relatively high number of cattle per 100 ha of agricultural land, compared to other areas of the country (Central Statistical Office of Poland, 2017). This part of Poland has the highest number of milk producers and milk processing plants. Thus, the utilization of SMS and cow slurry on arable land and pastures in this region would both reduce the potential negative impacts of these agricultural wastes and increase the availability of the dietary intake for cattle and other animal life in the region.

CONCLUSIONS

Cow slurry application to legume–grass mixtures increased the forage yield more than SMS. The content of NDF, ADF and ADL fractions in legume–grass mixtures was higher in the forage from plots with SMS than from plots with cow slurry. The mixture of orchard grass with alfalfa produced forage of the best quality with regard to the content of fiber fractions, mainly on plots with the highest dose of SMS combined with the lowest amount of cow slurry. From a practical point of view, to produce forage of good quality when it comes to NDF fiber content it is recommended to use 30 t ha⁻¹ of SMS with together with 20 m³ ha⁻¹ of cow slurry. In turn, with regard to high forage yields and high ADF and ADL content it is recommended to use slurry.

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REFERENCES


