

Research Article

The Effect of Spent Mushroom Substrate and Cow Slurry on Sugar Content and Digestibility of Alfalfa Grass Mixtures

E. Malinowska  and K. Jankowski

Siedlce University of Natural Sciences and Humanities, Faculty of Agrobioengineering and Animal Husbandry, Prusa 14 Street, 08-110 Siedlce, Poland

Correspondence should be addressed to E. Malinowska; elzbieta.malinowska@uph.edu.pl

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The aim of this paper is to evaluate the effect of different doses of spent mushroom substrate and cow slurry on sugar content and digestibility of hybrid alfalfa and grass mixtures. The main factors were different doses of organic material: mushroom substrate and slurry, and the following legume grass mixtures: M1-orchard grass (*Dactylis glomerata*), perennial ryegrass (*Lolium perenne*), and hybrid alfalfa (*Medicago x varia* T. Martyn); M2-orchard grass, hybrid alfalfa; M3-perennial ryegrass, hybrid alfalfa. In each growing season, the mixtures were harvested three times during three years of their full use. Sugar content and dry matter digestibility were determined with near-infrared spectroscopy (NIRS) using the NIRFlex N-500 spectrometer. Of all fertilizer treatments, the application of mushroom substrate at a dose of 20 t·ha⁻¹ in combination with 40 m³ of slurry resulted in the best forage quality with its highest digestibility. In the mixture of perennial ryegrass and hybrid alfalfa increasing doses of mushroom substrate with decreasing doses of slurry lowered soluble sugar content and digestibility.

1. Introduction

Legume grass mixtures provide one of the best balanced types of roughage [1–4]. Such mixtures are often used in organic production systems [5], being beneficial for successive plants in crop rotation [6].

A starting dose of mineral nitrogen used in the cultivation of these plants can be replaced with organic fertilizer or organic waste, such as slurry or mushroom substrate [7, 8]. The use of organic materials is necessary when organic matter content in the soil is low. About 45% of European soils have a low or very low content of organic carbon (0–2%). In Poland, 90% of agricultural soils in the layer of 0–25 cm contain 0–2% of organic carbon, i.e., below 3.5% of humus [9]. Research on agricultural use of mushroom substrate in east central Poland is extremely important because this region is undergoing intense development of mushroom production, significant not only in Poland but in

the whole of Europe. Application of organic materials in the cultivation of feed crops, that is, legume grass mixtures, not only can improve plant yields, but it also increases soil fertility.

Cantalapiedra-Hijar et al. [10] and Olszewska et al. [11] found that legume plants and their mixtures exhibited higher digestibility than grass grown on its own. Dry matter digestibility largely depends on the time of harvesting and decreases with plant aging [12], and it increases in consecutive harvests [13–15]. Many authors [16–18] reported that between 18 and 70% of nitrogen in grass grown with legumes comes from its fixing from the atmosphere by the latter plants. This way growing grass with legumes reduces costs and reduces the losses of nitrogen leached deeper into the soil [19].

The aim of the paper is to evaluate the content of sugars and digestibility of hybrid alfalfa and grass mixtures treated with different doses of spent mushroom substrate and cow slurry.

2. Materials and Methods

This paper is based on a three-year field experiment carried out between 2013 and 2015 and replicated three times. It was set up in the autumn of 2012 in a split-plot design, with plots of 3.0 m². The main experimental factors were different doses of mushroom substrate and cow slurry applied to legume grass mixtures. There were three experimental factors in the study: (1) treatment, with spent mushroom substrate and cow slurry used separately and in various combinations; (2) harvests/years; (3) legume grass mixtures.

There were the following units in the experiment:

- (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).

The slurry was collected from cows producing 6,000 to 8,000 liters of milk a year and housed indoors on straw bedding, with manure removed daily. Before seeds were planted, the mushroom was mixed with a 20–25 cm layer of the soil. The 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.

Three plant species were involved in the experiment: alfalfa hybrid of Tula variety, the Bora variety of orchard grass, and perennial ryegrass of Info variety. These species were grown as three legume grass mixtures, with an equal share of each component: M1-orchard grass, perennial ryegrass, alfalfa hybrid; M2-orchard grass, hybrid alfalfa; M3-perennial ryegrass, alfalfa hybrid. Taking into account different germination capacity the sowing rate of plants grown on their own was as follows (kg·ha⁻¹): hybrid alfalfa *Medicago x varia Martyn*—23; *Dactylis glomerata*—21; *Lolium perenne*—31. 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. The first date of harvesting mixtures was carried out at the stage of heading for grass, the second at the stage of beginning of flowering of alfalfa, and the third at full budding of alfalfa.

The experiment was founded on the soil of the anthropogenic order, culture Earth type, and the subtype of hortisol (Polish classification system), with the granulometric composition of loamy sand. Before the experiment started, the carbon content in soil organic compounds (Corg) was 13.50 g·kg⁻¹ DM, with total nitrogen content of 1.30 g·kg⁻¹ DM, while C : N ratio was 10.4 : 1, and pH was 6.8. The content of total nitrogen, phosphorus (P), and potassium (K) in the mushroom substrate was as follows: 24.50; 9.50; 13.20 g·kg⁻¹ DM, and in cow slurry it was 48.00; 12.64; 43.16 g·kg⁻¹ DM.

The total nitrogen content was determined by the Kjeldahl method, and the concentration of other chemical elements was measured by the ICP-AES method, after dry mineralization in a muffle furnace at 450°C. The organic carbon content in the soil was determined by the oxidation-titration method. In order to determine the temporal variation of meteorological elements and their effects on vegetation, Sielianinov's hydrothermal coefficient was calculated [20] on the basis of monthly sums of precipitation (P) and monthly sums of air temperature ($\sum t$), using the formula: $K = (P/0.1) \sum t$.

Optimum temperature and precipitation (Table 1) were only in April 2014 and in September 2015. In the remaining months of the growing periods, weather conditions varied from extremely dry in August 2015 to extremely wet in May 2013. The best conditions were at the beginning of each growing period. It can be concluded that the most difficult conditions for plant development were in 2015, when, apart from May and the end of the growing period, the weather varied from moderately dry to extremely dry.

Within three years of their full use, plants were harvested three times in each growing season. Each time soluble sugar content and dry matter digestibility were determined with near-infrared spectroscopy (NIRS), using the NIRFlex N-500 spectrometer.

The results of the research were processed statistically using three factor analysis of variance. Fisher's F-test was used to determine whether the impact of experimental factors was significant, while the value of the LSD_{0.05} was calculated with Tukey's test. The Statistica program, version 10.0 StatSoft, was applied for all other calculations [21].

3. Results

Sugar content in the mixtures varied depending on the treatment, growing season, and harvest (Tables 2 and 3). In regard to treatment, plants from plots where slurry was applied had the highest content of sugars (Table 2) of 64.81 g·kg⁻¹, while it was the lowest (54.68 g·kg⁻¹) in the forage from the control. Generally, mushroom substrate, slurry, and their mixture resulted in a statistically significant increase in sugar content in relation to the control.

Additionally, sugar content steadily increased in a statistically significant way throughout the experiment from 51.76 g·kg⁻¹ in the first year to 67.19 g·kg⁻¹ in the third one (Table 2). The plants responded to almost all fertilizer treatments with an increase in sugar content. The highest content was in the forage harvested in 2015, the last year of the experiment, on the plot where slurry was applied (77.19 g·kg⁻¹), with the lowest in 2013 in plants treated with 20 t of the mushroom substrate with 40 m³ of slurry (48.31 g·kg⁻¹).

Regarding the differences between mixtures, their sugar content (Table 3) was very similar. Statistically significant differences were only noted between the first mixture (orchard grass, perennial ryegrass, and hybrid alfalfa) and the third (perennial ryegrass and hybrid alfalfa). They contained, respectively, 59.36 and 58.36 g·kg⁻¹ of sugar. However, both mushroom substrate and slurry increased the average content of soluble sugars in all mixtures. The highest content

TABLE 1: 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)

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

TABLE 2: The effect of fertilizer on sugar content ($\text{g}\cdot\text{kg}^{-1}$ DM) in legume grass mixtures in consecutive growing seasons.

Mixture (C)	Year (B)	Treatment (A)						Mean
		0	SMS	CS	SMS ₁₀ + CS ₆₀	SMS ₂₀ + CS ₄₀	SMS ₃₀ + CS ₂₀	
Orchard grass + ryegrass + alfalfa	2013	48.67	52.24	78.13	60.50	50.32	46.82	56.11
	2014	48.78	49.01	59.31	57.59	62.47	55.50	55.44
	2015	59.34	61.20	80.93	63.75	68.30	65.62	66.52
Orchard grass + alfalfa	2013	49.11	53.83	50.98	51.43	46.13	51.88	50.56
	2014	56.78	62.22	50.71	62.78	51.03	68.90	58.74
	2015	66.25	70.21	68.67	71.52	63.47	66.02	67.69
Ryegrass + alfalfa	2013	47.67	50.36	52.09	51.06	48.49	41.94	48.60
	2014	55.33	56.67	60.49	60.71	62.24	59.21	59.11
	2015	60.24	62.69	81.97	66.65	66.13	66.47	67.36
Fertilizer effect		54.68	57.60	64.81	60.67	57.62	58.04	58.90
Growing season effect								
2013		48.48	52.14	60.40	54.33	48.31	46.88	51.76
2014		53.63	55.97	56.84	60.36	58.58	61.20	57.76
2015		61.94	64.70	77.19	67.31	65.97	66.04	67.19

LSD_{0.05} for: A = 1.54; B = 0.890; C = 0.890; B/C = 1.54; C/B = 1.54; C/A = 1.54; A/B = 2.66; A/C = 1.88; B/A = 2.18, SMS: spent mushroom substrate, CS: cow slurry.

TABLE 3: The effect of fertilizer on sugar content ($\text{g}\cdot\text{kg}^{-1}$ DM) in legume grass mixtures in consecutive harvests.

Mixture (C)	Harvest (cuts) (B)	Treatment (A)						Mean
		0	SMS	CS	SMS ₁₀ + CS ₆₀	SMS ₂₀ + CS ₄₀	SMS ₃₀ + CS ₂₀	
Orchard grass + ryegrass + alfalfa	I	53.11	55.85	66.37	51.49	56.37	54.37	56.26
	II	50.94	51.21	77.54	59.80	68.63	57.79	60.99
	III	52.73	55.40	74.46	70.55	56.09	55.78	60.83
Orchard grass + alfalfa	I	53.44	59.34	52.28	55.79	56.96	58.68	56.08
	II	57.11	61.00	62.83	65.06	49.25	60.40	59.28
	III	61.58	65.93	55.25	64.88	54.43	67.71	61.63
Ryegrass + alfalfa	I	49.44	53.12	66.79	58.32	53.99	51.37	55.51
	II	58.84	61.00	60.63	59.02	62.51	56.06	59.68
	III	54.95	55.58	67.13	61.08	60.36	60.20	59.88
Mixture effect								
Orchard grass + ryegrass + alfalfa		52.26	54.15	72.79	60.61	60.36	55.98	59.36
Orchard grass + alfalfa		57.38	62.09	56.78	61.91	54.55	62.26	59.00
Ryegrass + alfalfa		54.41	56.57	64.85	59.47	58.95	55.88	58.36
Harvest effect								
I		52.00	56.10	61.81	55.20	55.77	54.81	55.95
II		55.63	57.74	67.00	61.29	60.13	58.08	59.98
III		56.42	58.97	65.61	65.50	56.96	61.23	60.78

LSD_{0.05} for: A = 1.38; B = 0.802; C = 0.802; B/C = 1.39; C/B = 1.39; C/A = 1.39; A/B = 2.40; A/C = 1.70; B/A = 1.96, SMS: spent mushroom substrate, CS: cow slurry.

(72.79 $\text{g}\cdot\text{kg}^{-1}$) was in orchard grass with ryegrass and alfalfa grown on the plot with slurry, and lowest (54.41 $\text{g}\cdot\text{kg}^{-1}$) in ryegrass with alfalfa on the control.

There were differences in sugar content between consecutive harvests. As an average effect of both organic

fertilizers it gradually increased from the first harvest (55.95 $\text{g}\cdot\text{kg}^{-1}$) to the third one (60.78 $\text{g}\cdot\text{kg}^{-1}$).

It was found that dry matter digestibility was variable depending on the fertilizer, growing season, and harvest (Tables 4 and 5). Both mushroom substrate and slurry

TABLE 4: The effect of fertilizer on the digestibility of legume grass mixtures (%) in consecutive growing seasons.

Mixture (C)	Year (B)	Treatment (A)						Mean
		0	SMS	CS	SMS ₁₀ + CS ₆₀	SMS ₂₀ + CS ₄₀	SMS ₃₀ + CS ₂₀	
Orchard grass + ryegrass + alfalfa	2013	48.44	49.42	53.11	57.65	54.59	46.15	51.56
	2014	52.00	56.74	58.91	55.35	60.50	56.64	56.69
	2015	50.42	47.16	53.97	49.70	53.42	49.66	50.72
Orchard grass + alfalfa	2013	54.67	57.34	54.58	49.29	55.44	50.41	53.62
	2014	58.22	60.80	55.20	52.71	58.20	57.50	57.11
	2015	51.36	51.02	50.68	50.75	47.94	46.69	49.74
Ryegrass + alfalfa	2013	42.44	43.80	53.87	58.14	55.20	48.39	50.31
	2014	51.00	53.70	59.76	57.40	60.32	58.82	56.83
	2015	49.61	51.57	52.30	53.43	50.51	45.45	50.48
Fertilizer effect								
		50.91	52.39	54.71	53.82	55.13	51.08	53.01
Growing season effect								
2013		48.52	50.18	53.85	55.03	55.08	48.32	51.83
2014		53.74	57.08	57.96	55.15	59.68	57.65	56.88
2015		50.46	49.92	52.32	51.29	50.62	47.27	50.31

LSD_{0.05} for: A = 1.12; B = 0.648; C = 0.648; B/C = 1.12; C/B = 1.12; C/A = 1.12; A/B = 1.938; A/C = 1.37; B/A = 1.59, SMS: spent mushroom substrate, CS: cow slurry.

TABLE 5: The effect of fertilizer on digestibility of legume grass mixtures (%) in consecutive harvests.

Mixture (C)	Harvest (cuts) (B)	Treatment (A)						Mean
		0	SMS	CS	SMS ₁₀ + CS ₆₀	SMS ₂₀ + CS ₄₀	SMS ₃₀ + CS ₂₀	
Orchard grass + ryegrass + alfalfa	I	52.00	51.24	55.42	50.50	55.39	47.67	52.04
	II	49.37	48.43	56.77	58.44	59.05	53.97	54.34
	III	49.49	53.64	53.80	53.75	54.07	50.81	52.59
Orchard grass + alfalfa	I	54.22	55.17	50.46	45.54	53.34	44.32	50.51
	II	54.67	54.53	55.58	55.69	53.60	51.45	54.25
	III	55.36	59.46	54.41	51.51	54.64	58.83	55.70
Ryegrass + alfalfa	I	46.56	47.85	55.88	59.35	53.15	49.10	51.98
	II	53.59	55.50	53.38	52.16	59.26	51.15	54.17
	III	42.91	45.73	56.68	57.46	53.62	52.41	51.47
Mixture effect								
Orchard grass + ryegrass + alfalfa		50.29	51.10	55.33	54.23	56.17	50.82	52.99
Orchard grass + alfalfa		54.75	56.39	53.48	50.92	53.86	51.53	53.49
Ryegrass + alfalfa		47.68	49.69	55.31	56.32	55.34	50.89	52.54
Harvest effect								
I		50.93	51.42	53.92	51.80	53.96	47.03	51.51
II		52.54	52.82	55.24	55.43	57.31	52.19	54.25
III		49.26	52.94	54.96	54.24	54.11	54.02	53.25

LSD_{0.05} for: A = 3.02; B = 1.75; C = n.s.; B/C = 3.03; C/B = 3.03; C/A = 3.03; A/B = 5.23; A/C = 3.70; B/A = 4.29, n.s.: not significant; SMS: spent mushroom substrate, CS: cow slurry.

application affected dry matter digestibility in a statistically significant way. The highest (55.13%) was in plants from the plot treated with 20 t of the mushroom substrate and 40 m³ of slurry, while the lowest (50.91%) was on the control.

As regards differences between growing seasons (Table 4), average digestibility was the highest (56.88%) in plants harvested in 2014, the second year, while the lowest (50.31%) was noted in the third year. Analyzing fertilizer effect in different growing seasons (Table 4), it was found that the highest digestibility (59.68%) was exhibited by the forage in the second year on the plot with 20 t of the mushroom substrate and 40 m³ of slurry. Plants from the plot on which 30 tons of mushroom substrate and 20 m³ of slurry were applied in the third year had the lowest

digestibility (47.27%). In relation to the control slurry application, each year resulted in an increase in plant digestibility.

The highest digestibility over the three growing seasons (Table 5) was 53.49% for the mixture of orchard grass with alfalfa, with the lowest for ryegrass with alfalfa (52.54%). The differences between dry matter digestibility of different mixtures were not statistically significant. Regarding the effect of organic fertilizer (Table 5), orchard grass with hybrid alfalfa had the highest digestibility of all mixtures (56.39%) on the plot with the mushroom substrate, and the lowest (47.68%) was the mixture of perennial ryegrass with hybrid alfalfa on the control. The mixture of orchard grass with alfalfa had better digestibility when it was treated with mushroom substrate than with slurry, while for the mixture

of ryegrass and alfalfa it was the opposite, with plants having higher digestibility on the plot with slurry.

Digestibility varied between individual harvests (Table 5) with the highest (54.25%) in the forage from the second cut. In the first harvest it was the lowest (51.51%) differing significantly from the forage of the second and third harvests.

4. Discussion

The mixtures of alfalfa with grass have higher and more stable yields and better nutritional value as fodder than the same plants grown in monoculture [22]. In the present experiment, organic fertilizer raised soluble sugar content. Similar results were observed by Wróbel et al. [23], who demonstrated a slight increase in sugar content in their experiment. Czyż et al. [24] found that this content varied between different mixtures and the differences were statistically significant. According to the latter authors, sugar content is affected by plant development stage at which they are harvested.

According to Dembek and Łyszczarz [25] ryegrass and orchard grass grown on their own contained about $100 \text{ g}\cdot\text{kg}^{-1}$ of sugar. Godlewska and Ciepela [26] found that the same plant species contained, on average, $73.7 \text{ g}\cdot\text{kg}^{-1}$ of soluble sugars, while in the case of *Festulolium braunii* it was much higher, amounting to $130.9 \text{ g}\cdot\text{kg}^{-1}$. In general, *Fabaceae* plants have lower amounts of soluble sugars than grass [27]. However, legumes contain essential nutrients for livestock, being rich in protein, vitamins, and minerals. Compared with grass species they have high content of crude protein, low content of carbohydrates, and high buffer capacity, which is why legumes are difficult to conserve as silage [28, 29].

Sugar content in plants from different harvests is affected, among other factors, by air temperature in individual months. With rising air temperature, plant respiration increases, requiring higher amounts of sugar [30].

Digestibility is a measure of how much forage is absorbed from the gastrointestinal tract into the body. According to Stachowicz [31], roughage intended for feeding ruminants should have digestibility at around 65%. The forage in the present experiment had somewhat lower digestibility.

The present results with dry matter digestibility of alfalfa mixtures with grasses, being the highest in the second year and lowest in the third, were confirmed by Gaweł [14]. Slightly higher digestibility than in the present experiment (about 60.3%) was exhibited by the mixture of alfalfa with *Festulolium braunii* in an experiment conducted by Sosnowski [2]. The same author [2] and Borowiecki [32] demonstrated that to a large extent, forage digestibility is affected by the share of alfalfa in a mixture. Sosnowski [2] reported that out of three legume grass mixtures harvested three times a year, red clover with *Festulolium braunii* had much higher digestibility than alfalfa with *Festulolium braunii*.

The present experiment showed a significant effect of fertilizer treatment on dry matter digestibility, which again

was confirmed by Sosnowski [2]. Small differences in digestibility between different mixtures containing grasses, alfalfa, or other *Fabaceae* plants were reported by Gaweł [33]. However, it was generally higher than in the present experiment. It can be concluded that other *Fabaceae* plants, like *Onobrychis viciifolia* or *Lotus corniculatus* L., added to alfalfa and grass mixtures increase forage digestibility.

Plant digestibility is also affected by the length of a growing cycle, that is, the number of harvests in the growing period, which is closely related to the development stage at which crops are harvested, but also by the pace of plant senescence [12, 13]. In his experiment Gaweł [14] found that dry matter digestibility of legume grass mixtures harvested five times was as high as 80%.

5. Conclusions

Both soluble carbohydrate content and dry matter digestibility indicate that the response of individual legume grass mixtures to fertilizer treatments was very diverse.

Of all fertilizer combinations, forage with the highest digestibility was obtained as an effect of the application of $20 \text{ t}\cdot\text{ha}^{-1}$ of the spent mushroom substrate with 40 m^3 of cow slurry.

In the mixture of ryegrass and alfalfa increasing doses of the mushroom substrate and decreasing doses of cow slurry lowered soluble sugars content and digestibility.

The slurry in comparison with mushroom substrate increased the content of soluble sugars in legume grass mixtures, as well as their digestibility.

Data Availability

The results of sugar content and stability of grass mixtures used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare no conflicts of interest.

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