Changes in the Nutritional Value of Timothy (*Phleum pratense* L.) Biomass as a Response to Varied Organomineral Treatment

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Abstract

The aim of the experiment was to determine the impact of the interaction of slurry with soil conditioners used in organic farming on selected feed parameters of Phleum pratense biomass. The research was conducted in three replications at the experimental facility of Siedlce University (52°10'03"N; 22°17'24"E, Poland) in years 2015 - 2017. The fertilizers used in the experiment were cattle slurry used separately and supplemented with leonardite extract and vermicompost extract, as well as mineral NPK. Cattle slurry was used each year at a total dose of 30 m3 ha-1, divided into individual cuts. Soil conditioners were used in accordance with the manufacturer's recommendations. NPK mineral fertilization was applied at doses: N – 100, P – 30, K – 100 kg ha-1. The tested plant in the experiment was forage grass of the species Phleum pratense of the Secesja variety. The content of the neutral detergent fiber fraction and acid detergent fiber fraction was analyzed in the plant material samples using near-infrared reflectance spectroscopy (NIRS). The relative nutritional value was determined based on the neutral and acid detergent fiber fraction content. It was shown that the addition of vermicompost and NPK extract to the slurry improved the values of the tested parameters compared to the slurry alone. The feed value of biomass collected from facilities fed with slurry and NPK was higher than the feed value of biomass fertilized with slurry with leonardite extract, but comparable to the value of biomass fertilized with slurry supplemented with vermicompost extract.

Introduction

Consisting of structural carbohydrates, fiber is a very significant parameter of feed rations. It is not only a source of energy, but it also regulates digestion processes and ensures proper gastrointestinal motility[1]. Structural carbohydrates, the building blocks of plant cell walls, include neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL), the latter being the least digestible[2, 3, 4]. Many authors[5, 6, 7, 8] argue that roughage qualitative assessment should be based on NDF and ADF content rather than on crude fiber content, or simultaneously with it. In the diet of ruminants, neutral detergent fiber is particularly important, filling the rumen, being a source of energy for microorganisms living in it and giving the forage the desired structure.

However, if NDF and ADL fiber content in forage is too high, it impairs its digestibility and results in its lower utilization and intake by animals. According to Van Soest et al.[9], components of cell walls, i.e. cellulose, hemicellulose and lignin, determined as NDF and ADF, limit forage intake, digestibility and energy value. There is an important relationship between the digestibility and the intake of dry matter and its NDF content, which is used to estimate forage energy value [10]. Based on ADF and NDF content, it is possible to comprehensively assess forage quality using the relative feed value (RFV) index, which combines digestibility and feed intake into a single parameter[11].

One of the main advantages of manure and slurry, justifying their widespread use, is that they contain practically all the nutrients necessary for the proper growth and development of plants. The only problem is that the proportions of their nutrients are not always right from the point of view of plant nutrition. In addition, according to studies on slurry chemical composition, a significant part of its nitrogen (approx. 50%) is ammonium nitrogen, hence the risk of its losses in the form of ammonia or nitrates[12, 13, 14]. When slurry is used on light soils, quite a lot of potassium leaching is observed. For this reason, it is necessary to supplement losses of all those plant nutrients with mineral fertilizers.

However, due to high costs, organic farming increasingly uses mineral-organic substances as fertilizers, which include soil conditioners. Increasing soil buffering capacity and water holding capacity, they have many advantages. Moreover, they have chelating abilities, which results in a more rational use of macro and micronutrients by plants and in a reduction of nutrient losses. Research by Wiśniewska-Kadżajan and Jankowski[15, 16], Sabry and Abdal-Latife[17], Wiśniewska-Kadżajan and Stefaniak[18], Godlewska and Ciepiela[3], Bozhanska[19] and Truba et al.[20] on the agricultural use of similar products and fertilizers has shown that they have a beneficial effect on the yield and chemical composition of grassland vegetation.

Forage grasses grown both on permanent and alternating grassland include *Phleum pretense*, a very valuable species. It is a nitrophilic plant and with good nitrogen nutrition it grows quickly and produces high energetic yields with good nutritional value and good digestibility and palatability[21, 22] [Bélanger et al., 2001; Radkowski et al., 2020]. However, it is not a competitive species and due to its shallow root system it is sensitive to periodic droughts[23], but it is frost resistant[24].

The aim of the research was to determine the impact of the interaction between slurry and leonardite extract, and slurry with vermicompost extract, on *Phleum pratense* selected nutritional parameters.

Material and methods

The experiment was carried out in years 2015–2017 field experiment established on the University in Siedlce experimental plot (Poland 52^{o}10'N, 22^{o}17'E), with three replications and the plots about dimensions 1.5 × 3.0 m on soil with a granulometric composition of loamy sand included in the order of anthropogenic soils, culture-earth type and hortisole subtype[25].

The fertilizing materials used in the experiment were cattle slurry from dairy cows, used separately and supplemented with vermicompost and leonardite extracts as well as with mineral NPK fertilizers.

The following research objects were separated in the experiment:

- control (without fertilization) (O);
- slurry (S);
- slurry + leonardite extract (S + LE) ;
- slurry + vermicompost extract (S + VE);
- slurry + mineral NPK (S + NPK).

Some physicochemical properties of slurry and soil are presented in Table 1.

Table 1
Selected physicochemical properties of slurry and soil

Parameter	Unit	Contents	
		Slurry	Soil
pH	-	7.30	6.50
C:N ratio		8:1	11:1
C organic	$g\ kg^{-1}$ s.m.	248.0	14.5
N total		31.0	1.30
P total		7.10	0.85
K total		32.5	1.32
P – $H_2PO_4^-$ available	$mg\ kg^{-1}$ s.m.	-	6.03
K – K^+available		-	12.03
Mg – Mg^{2+} available		-	6.50

Slurry was applied each year at a total dose of 30 $m^3\ ha^{-1}$ in three equal parts, in the spring and after the first and second harvest, in accordance with the guidelines contained in the Nitrates Directive and the Code of Good Agricultural Practice[26, 27].

The soil conditioners used are an extract from manure vermicomposts with the trade name Humus Active and an extract from leonardite, a mineral mined in brown coal mines produced under the name Rosahumus. According to the Institute of Cultivation, Fertilization and Soil Science in Puławy, vermicompost extract and leonardite extract are soil conditioners and can be used in organic agriculture[28]. The chemical composition of vermicompost extract, is 56% of organic matter, including 85% of humic acids, approx. 100 g kg^{-1} of K and 5000 mg kg^{-1} of Fe. The leonardite extract contains of useful microorganisms (PM), as well as 55% of humic acids, including humin acids, fulvic acids as well as humins and ulmins. It also contains NPK in the following amounts: 0.2; 1.3; 3.0 g kg^{-1} and 450 mg kg^{-1} Fe. Leonardite extract was applied at 3 kg ha^{-1}, while vermicompost extract at 20 L ha^{-1}. NPK mineral fertilization was applied in doses: 100, 30 and 100 kg ha^{-1}. All phosphorus was used before the vegetation started, while nitrogen and potassium were divided into three doses for each regrowth. Fertilization was tested on *Phleum pretense*, a fodder grass of the Secesja variety, planted in autumn 2014. Three cuts of grass were collected each year between 2015 and 2017. During harvesting, 0.5 kg of fresh weight was collected from each plot to determine dry weight and perform chemical analyses.

In appropriately prepared samples, the content of neutral detergent fiber and acid detergent fiber fractions was determined by near-infrared reflectance spectroscopy (NIRS) using the NIRFflex N-500 apparatus[29, 30].

Using the following formulas developed by Linn and Martin[11], digestibility (DDM), dry matter intake (DMI) and relative nutritional value (RFV) were calculated based on the NDF and ADF fraction content. Based on the RFV ranges given by Linn and Martin[11], the feed was classified into appropriate quality classes useful in feeding various groups of ruminants. (Table 2).

RFV = (DDM x DMI) : 1.29

where:

RFV - relative feed value (dimensionless quantity),

DDM (digestible dry matter) = 88.9–0.779 x ADF (%),

DMI - dry matter intake = 120 : NDF (% of body weight).

Table 2

Suitability of forage in animal nutrition based on RFV[11] [Linn and Martin 1989]

Quality class	**RFV ranges**	**Feeding groups**
I	> 151	Best cows with high productivity
II	125–151	Good cows and young heifers selected for mating
III	103–124	Good cattle for fattening, older heifers, marginally for dairy cows
IV	87–102	Fattening or dried dairy cows
V	75–86	Fattening dry cows requiring high-energy forage

The results of plant material evaluation were developed statistically using the analysis of variance for a three-factor experiment. The significance of differences between means was estimated with Tukey’s test at the significance level $\alpha \le 0.05$. Statistica 12.0 was used for the calculations [31].

Meteorological data for the years of experiment were obtained from Hydrological and Meteorological Station in Siedlce (Table 3).

Table 3
Average monthly air temperature (°C) and monthly rainfall (mm) in the years of study

Year	Month							
	IV	V	VI	VII	VIII	IX	X	Średnia
	Temperature (°C)							
2015	9.7	13.7	15.1	20.5	17.8	13.7	8.4	14.1
2016	8.2	12.3	16.5	18.7	21.0	14.5	6.5	14.0
2017	6.9	13.9	17.8	16.9	18.4	13.9	9.0	13.8
Means	8.3	13.3	16.5	18.7	19.1	14.0	8.0	13.9
Multiannual means	8.5	14.0	17.4	19.8	18.9	13.2	7.9	14.2
	Precipitation (mm)							
2015	39.5	79.5	74.2	37.5	105.7	26.3	3.0	52.2
2016	30.0	100.2	43.3	62.6	11.9	77.1	39.0	52.0
2017	59.6	49.5	57.9	23.6	54.7	80.1	53.0	54.1
Means	43.0	76.4	58.5	41.2	57.4	61.2	31.7	52.8
Multiannual means	33.0	52.0	52.0	65.0	56.0	48.0	28.0	47.7

Ethical approval

The authors hereby declare that all methods were carried out in accordance with relevant guidelines.

Results

Neutral Detergent Fiber (NDF)

Phleum pratense neutral detergent fiber content (Table 4) significantly varied depending on fertilizer treatment, years of research and harvests. The differences in NDF content between plants from the control plot and those treated with slurry on the one hand and those treated with slurry used with soil conditioners or with mineral NPK on the other were statistically significant. The highest value (459.4 g kg^{-1}) was recorded in plants from the plot with slurry used together with leonardite extract, while the lowest (402.4 g kg^{-1}) from the control plot. The interaction of slurry with the leonardite extract resulted in an increase by approx. 5% in the amount of NDF fraction in relation to plants treated only with slurry.

Forage collected in the third growing period (2017) contained the most NDF (442.6 g kg^{-1}), while the lowest amount (425.1 g kg^{-1}), about 4% lower, was collected in the first (2015) year of research.

Additionally, a significantly difference was recorded between the second and third year. When considering harvests, the highest (437.8 g kg^{-1}) NDF amounts were found in the third one, while the lowest in the first (421.7 g kg^{-1}). The biomass of the first and second harvests also differed significantly in NDF fraction content.

Table 4. Content of neutral detergent fiber (NDF) in *Phleum pratense* biomass (g kg^{-1}DM)

Years	Regrowth	Fertilization					Mean
		O*	S	S + VE	S + LE	S + NPK	
2015	I	449.8	435,9	398.7	455.9	385.8	425.2
	II	460.4	450.7	372.8	469.2	376.8	426.0
	III	458.6	433.9	386.9	476.4	365.4	424.2
	Mean	456.3 ab**	440.2 b	386.1 c	467.2 a	376.0 c	425.1 B***
2016	I	452.8	395.8	385.4	425.7	392.4	410.4
	II	437.0	436.5	425.5	433.5	410.5	430.6
	III	462.3	421.8	432.8	440.5	435.2	436.5
	Mean	447.4 a	411.4 b	414.6 b	443.2 a	412.7 b	425.8 B
2017	I	423.5	452.8	426.5	457.9	386.5	429.4
	II	434.5	462.3	446.8	460.4	424.0	445.6
	III	430.0	470,5	432.9	485.2	445.2	452.8
	Mean	429.3 b	461.9 a	435.4 b	467.8 a	418.5 b	442.6 A
Mean		444.3 a	437.8 b	412.0 c	459.4 a	402.4 c	
Mean across regrowths							
I		442.0	428.2	403.5	446.5	388.2	421.7 B
II		444.0	443.2	415.0	464.4	403.8	434.1 A
III		447.0	442.1	417.5	467.4	415.3	437.8 A

*O - control, S – slurry, S + VE - slurry + vermicompost extract, S + LE - slurry + leonardite extract, S + NPK - slurry + mineral NPK;

**The means in the lines marked with the same small letters do not differ significantly at $p \le 0.05$;

***The means in the columns marked with the same capital letters do not differ significantly at $p \le 0.05$.

On the plots with slurry applied together with vermicompost ekstract or NPK fertilizers, the content of NDF significantly varied between the first year and both the second and the third. Biomass from slurry-only plots showed a significant difference between the third year on the one hand and the first and second on the other. A significant variation was also noted between plants treated with slurry with leonardite extract and those treated with slurry supplemented vermicompost extract or NPK fertilizers. In the second year of research, different amounts of NDF were observed between the plot where slurry was applied together with leonardite extract and plots treated with slurry alone, slurry with the vermicompost extract or with NPK. A similar difference was recorded between biomass from the control plot and plants treated with slurry alone or slurry applied with vermicompost extract or with NPK fertilizers. In the third year, NDF content varied between control plants and those treated with slurry on its own or slurry applied together with leonardite extract, but also between the slurry treated plot and plots with slurry and vermicompost extract or NPK fertilizers and, additionally, between plants treated with slurry used with leonardite extract and those fertilized with slurry used with vermicompost extract or NPK fertilizers.

Acid Detergent Fiber (ADF)

Acid detergent fiber (ADF) content (Table 5) in *Phleum pratense* significantly varied across fertilizer treatment, years of research and harvests. Significant differences were noted between control plants and those treated with slurry supplemented with mineral fertilizers, but also between those treated with slurry on its own and treated with slurry applied with vermicompost extract or NPK fertilizers. The highest ADF content (293.9 g kg^{-1}) was recorded in biomass collected from the slurry treated plot, while the lowest (271.7 g kg^{-1}) in plants from the one with slurry and NPK fertilizers.

Table 5. Content of acid detergent fiber (ADF) in *Phleum pratense* biomass (g kg^{-1}DM)

Years	Regrowth	Fertilization					Mean
		O*	S	S + VE	S + LE	S + NPK	
2015	I	267.5	305.6	276.0	310.2	265.3	284.9AB***
	II	276.3	298.6	274.6	286.4	270.3	281.2 B
	III	295.2	321.5	280.8	305.5	276.8	296.0 A
	Mean	279.7 b**	308.6 a	277.1b	300.7 a	270.8 b	287.4 A
2016	I	280.5	270.5	284.6	268.6	267.5	274.3 B
	II	293.5	296.2	295.3	265.4	278.1	285.7 B
	III	318.6	307.6	285.9	315.2	280.6	301.6 A
	Mean	297.5 a	291.4 ab	288.6 ab	283.1ab	275.4 b	287.2 A
2017	I	272.7	262.3	260.5	268.7	262.7	265.4 B
	II	282.3	287.5	275.1	264.6	265.9	275.1 AB
	III	314.7	295.5	285.5	308.3	278.4	296.5 A
	Mean	289.9 a	281.8 ab	273.7 ab	280.5ab	269.0 b	279.0 B
Mean		289.0 ab	293.9 a	279.8 bc	288.1ab	271.7 c	
Mean across regrowths							
I		273.6 B	279.5 B	273.7 A	282.5 B	265.2 A	274.9 B
II		284.0 B	294.1AB	281.7 A	272.1 B	271.4 A	280.7 B
III		309.5 A	308.2 A	284.07 A	309.7 A	278.6 A	298.0 A

*O - control, S – slurry, S + VE - slurry + vermicompost extract, S + LE - slurry + leonardite extract, S + NPK - slurry + mineral NPK;

**The means in the lines marked with the same letters do not differ significantly at $p \leq 0.05$;

***The means in the columns marked with the same capital letters do not differ significantly at $p \leq 0.05$.

Significantly different content was found between the third year of research and both the first and second. When considering individual harvests, the highest (298.0 g kg^{-1}) ADF content was found in the third one and the lowest in the first (274.9 g kg^{-1}). There was a significant difference between the biomass of the second and third harvest. Additionally, a significant difference was recorded between the first and both the second and the third year in the biomass from the control plot.

In the first year ADF content in control plants was significantly different from both biomass from plots treated with slurry alone and slurry supplemented with leonardite extract. The content of ADF also differed significantly between plants treated only with slurry on its own or with slurry supplemented with leonardite extract on the one hand and biomass treated with slurry applied with vermicompost extract or NPK fertilizers on the other. In the second and third years, a significant difference in ADF was noted between control plants and those treated with slurry supplemented with NPK fertilizers.

Plants from the control plot and those treated with slurry and the leonardite extact contained significantly different ADF content in both the first and second harvests and the third one. A similar difference was noted between the first and third harvests in biomass from the plot treated with slurry alone. In the first and second harvests significant differences were observed between the control plot and the one treated with slurry applied together with vermicompost extract or NPK fertilizers. A similar difference was noted between plants treated with slurry supplemented with leonardite extract and plants treated with slurry supplemented with vermicompost or NPK fertilizers. In the biomass of the first harvest a difference in ADF fiber content was noted between *Phleum pratense* treated with slurry on its own and plants treated with slurry supplemented with vermicompost extract or NPK fertilizers. The results also proved differences in ADF fiber content between harvests. Significant differences were noted between the third and both the second and first harvest in the second and third year and between the second and third harvest in the first year.

Dry Matter Intake (DMI)

Dry matter intake (Table 6) of *Phleum pratense* significantly varied across fertilizer treatment, years of research and harvests. Significantly different values were recorded between control plants and those treated only with slurry on the one hand and those treated with slurry supplemented with Humus Active soil conditioner or mineral NPK fertilizers on the other. The highest DMI value (2.99%) was recorded for biomass from the plot with slurry and NPK fertilizers, while the lowest (2.61%) for plants treated with slurry supplemented with leonardite extract. The interaction of slurry with the vermicompost extract or with NPK fertilizers resulted in an increase in dry matter intake compared to biomass treated with slurry on its own by approx. 6% and 8%, respectively.

Table 6. Dry matter intake (DMI) of *Phleum pratense* (% body weight)

Years	Regrowth	Fertilization					Mean
		O*	S	S + VE	S + LE	S + NPK	
2015	I	2.67	2.75	3.01	2.63	3.11	2.83
	II	2.61	2.66	3.22	2.56	3.18	2.85
	III	2.62	2.76	3.10	2.52	3.28	2.86
	Mean	2.63b**	2.73b	3.11a	2.57b	3.19a	2.85A***
2016	I	2.65	3.03	3.11	2.82	3.06	2.93
	II	2.74	2.88	2.82	2.59	2.92	2.79
	III	2.65	2.84	2.77	2.72	2.76	2.75
	Mean	2.68a	2.92a	2.90a	2.71a	2.91a	2.82 A
2017	I	2.83	2.65	2.81	2.62	3.10	2.80
	II	2.76	2.59	2.68	2.61	2.83	2.69
	III	2.79	2.55	2.77	2.47	2.69	2.66
	Mean	2.79ab	2.60ab	2.76ab	2.57b	2.88a	2.72 B
Mean		2.70b	2.75b	2.92a	2.61b	2.99a	
Mean across regrowths							
I		2.72	2.81	2.98	2.69	3.09	2.86 A
II		2.70	2.71	2.91	2.58	2.98	2.78 AB
III		2.69	2.72	2.88	2.57	2.91	2.75 B

*O - control, S – slurry, S + VE - slurry + vermicompost extract, S + LE - slurry + leonardite extract, S + NPK - slurry + mineral NPK;

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Across growing periods, a significant variation in dry matter intake was found between the third year in relation to the second and first one. Considering the harvests, the highest DMI value (2.86%) was found for the first one, and the lowest for the third (2.75%). A significant difference between the first and both the second and third year of research was noted in biomass from the control and in that from the slurry-treated plot supplemented with the leonardite extract. Biomass from control and slurry-only plots showed a significant difference between the first and second year.

In the first year of research DMI values for control plants and those treated only with slurry were significantly different from those for *Phleum pratense* treated with slurry supplemented with the vermicompost extract or NPK mineral fertilizers. Significantly different DMI values were also found between plants treated with slurry applied with the leonardite extract and those treated with slurry supplemented with vermicompost extract or NPK fertilizers. In the third year of the experiment, the differences of this parameter were observed between the control plot and those treated with slurry supplemented with vermicompost extract or leonardite extract.

Dry Matter Digestibility (DDM)

Dry matter digestibility (Table 7) of *Phleum pratense* significantly varied in response to fertilizer treatment, years of research and harvests. Compared to the control plot and to those treated with slurry alone and slurry supplemented with vermicompost extract, significantly different digestibility was noted on the plot treated with slurry applied together with NPK fertilizers. The most digestible (67.7%) was the biomass collected from the plot treated with slurry supplemented with NPK fertilizers, while the least (66.0%) from the plot treated with slurry alone. Across growing periods, values between the third one and both the first and second were significantly different. When considering harvests, the biomass of the third harvest was significantly the least digestible (65.7%), while the most from the first (67.5%). The biomass of the second and third harvest also differed significantly in digestibility.

Table 7. Dry matter digestability (DDM) of *Phleum pratense* (%)

Years	Regrowth	Fertilization					Mean
		O*	S	S + VE	S + LE	S + NPK	
2015	I	68.1	65.1	67.4	64.7	68.2	66.7
	II	67.4	65.6	67.5	66.6	67.8	67.0
	III	65.9	63.8	67.0	65.1	67.3	65.8
	Mean	67.1a	64.9 b	67.3 a	65.5 b	67.8 a	66.5B***
2016	I	67.0	67.8	66.7	68.0	68.1	67.5
	II	66.0	65.8	65.9	68.2	67.2	66.6
	III	64.0	64.9	66.6	64.3	67.0	65.4
	Mean	65.7 b**	66.2 ab	66.4 ab	66.8 ab	67.4 a	66.5 B
2017	I	67.6	68.5	68.6	68.0	68.4	68.2
	II	66.9	66.5	67.5	68.3	68.2	67.5
	III	64.4	65.9	66.6	64.9	67.2	65.8
	Mean	66.3 b	66.9 ab	67.6 ab	67.0 ab	67.9 a	67.2 A
Mean		66.4bc	66.0c	67.1 ab	66.4 bc	67.7 a	
Mean across regrowths							
I		67.6 A	67.1 A	67.6 A	66.9 A	68.2 A	67.5 A
II		66.8 A	66.0 A	66.9 A	67.7 A	67.7 A	67.0 A
III		64.8 B	64.9 B	66.8 A	64.8 B	67.2 A	65.7 B

*O - control, S – slurry, S + VE - slurry + vermicompost extract, S + LE - slurry + leonardite extract, S + NPK - slurry + mineral NPK;

**The means in the lines marked with the same small letters do not differ significantly at $p \leq 0.05$;

***The means in the columns marked with the same capital letters do not differ significantly at $p \leq 0.05$.

Across the years of research, a significant difference between the first and both the second and third year was noted for control plants. *Phleum pratense* digestibility in the first year significantly varied between control plants on the one hand and those from plots treated with slurry alone and those treated with slurry supplemented with the vermicompost extract or NPK fertilizers on the other. A significant variation was also noted between the plot treated only with slurry and that on which slurry was supplemented with vermicompost extract or mineral fertilizers. There was a significant difference between plants supplied with slurry combined with the leonardite extract on the one hand and those treated with slurry applied with vermicompost extract or NPK fertilizers on the other. In the second and third years, a significant difference was noted between the control plot and that treated with slurry and NPK fertilizers.

There was a difference for plants of both the first and second harvest and the third on the control plot, the one treated only with slurry on its own and the one were slurry was applied with the leonardite extract. In the third harvest a significant difference between the control plot and the plot treated only with slurry on the one hand and plots treated with slurry supplemented with vermicompost extract or leonardite extract or NPK fertilizers on the other was noted.

Relative Feed Value (RFV)

The relative nutritional value (Table 8) of *Phleum pratense* significantly varied across fertilizer treatment, years of research and harvests. Values recorded for plants from the control plot and for that treated only with slurry were different to those for biomass fertilized with slurry supplemented with vermicompost extract or mineral fertilizers. The highest relative feed value (157.2) was noted for the biomass collected from the plot treated with slurry supplemented with NPK, while the lowest (134.8) for the biomass from the plot treated with slurry and leonardite extract. A significant variation was recorded between the plot treated with slurry and the leonardite extract and plots treated with slurry supplemented with vermicompost extract or mineral fertilizers.

Table 8. Relative nutritional value (RFV) of *Phleum pratense* biomass

Years	Regrowth	Fertilization					Mean
		O*	S	S + VE	S + LE	S + NPK	
2015	I	140.7	138.9	157.2	132.1	164,5	146.7A***
	II	136.1	135.5	168.5	132.0	167,5	147.9 A
	III	133.7	136.9	161.1	127.1	171,4	146.0 A
	Mean	136.8b**	137.1 b	162.3 a	130.4 b	167,8 a	146.9 A
2016	I	137.7	159.4	161.1	148.5	161,3	153.6 A
	II	140.6	147.0	144.1	136.9	152,4	144.2 B
	III	131.8	143.2	143.2	135.9	143,3	139.5 B
	Mean	136.7 b	149.9 ab	149.4 ab	140.4 ab	152,3 a	145.8 AB
2017	I	148.6	140.6	149.6	138.1	164,7	148.3 A
	II	143.2	133.8	140.5	138.0	149,6	141.0 AB
	III	139.3	130.2	143.2	124.4	140,4	135.5 B
	Mean	143.7 ab	134.9 ab	144.4 ab	133.5 b	151,6 a	141.6 B
Mean		131.9 b	140.6 b	152.1a	134.8 b	157,2a	
Mean across harvests							
I		142.4	146.3	156.0	139.6	163,5	149.5 A
II		140.0	138.8	151.0	135.6	156,5	144.4 B
III		134.9	136.8	149.2	129.1	151,7	140.3 B

*O - control, S – slurry, S + VE - slurry + vermicompost extract, S + LE - slurry + leonardite extract, S + NPK - slurry + mineral NPK;

**The means in the lines marked with the same small letters do not differ significantly at p ≤ 0.05;

***The means in the columns marked with the same capital letters do not differ significantly at p ≤ 0.05.

Forage RFV between both the first and second year and third one was significantly different. Across harvests, the highest RFV value (149.5) was recorded for the first one, while the lowest for the third (140.3). Similar significantly different values were recorded for the second and third harvest.

A significant variation across the years of research was noted between the second and third year for control plants and those treated with slurry alone. The biomass from the control plot and that from the slurry and vermicompost extract treated plot had significantly different RFV values between the second and both the first and third years. The values from the slurry and leonardite extract treated plot significantly varied between the first and second year of the experiment.

In the first year the RFV significantly varied between plants from the control plot and those treated with slurry alone on the one hand and those supplied with slurry and vermicompost extract or NPK fertilizers on the other, and, additionally, between plants from the plot where slurry was applied with the leonardite extract and plots where slurry was supplied with vermicompost extract or mineral NPK. In the second and third year, significant variation in RFV values of the biomass was noted between the control plot and that treated with slurry applied with the leonardite extract or NPK fertilizers. RFV values varied across harvests in research years. Significant differences were recorded between both the first and second harvest and the third one in the second year and between the first and third harvest in the third year. Significant differences for the third harvest were noted between the first and third year.

Discussion

Currently, mainly the content of neutral (NDF) and acid detergent fiber (ADF) is used to assess roughage quality. For ruminants, neutral detergent fiber is particularly important as it is a source of energy for rumen microorganisms and also gives feed the structure and provides ballast filling the rumen[2, 3]. In addition, NDF content makes it possible to predict daily dry matter intake[32]. In the present research, NDF content in *Phleum pratense* was similar to the values reported by Mertens[33], according to whom the desired amounts in hay should oscillate around 400 g kg^{-1} but according to Linn and Martin[11], the percentage of NDF should be 19–20%. In the present research an increase in NDF fiber was recorded in response to slurry supplemented with soil conditioners or NPK fertilizers. In contrast, Szparaga et al.[34] observed a decrease in NDF content in response to organic fertilizers in the form of slurry and manure.

Like lignin, silica and certain kinds of cellulose, acid-detergent fiber (ADF) is one of the least digestible forage ingredients. Its content should be about 200 g kg^{-1} as higher amounts results in the deterioration of forage digestibility [11]. In the present studies, ADF content oscillated from approx. 270 to 290 g kg^{-1} and was lower than those reported by Tomic et al.[35], who in *Dactylis glomerata* found its amounts exceeding 370 g kg^{-1}. In the present study, the application of slurry together with both extracts or NPK fertilizers increased ADF content compared to control plants and those treated with slurry applied on its own. In their research Cheng et al.[36] confirmed that the content of NDF and ADF depends on the development stage of the grass and on weather conditions during the growing period. This was confirmed in the present studies, in which the highest NDF was recorded during the third (2017) growing period, in which dry months prevailed. In turn, the highest ADF content in *Phleum pratense* was found in the second and third growing period (2015 and 2016). Both of them were not conducive to plant growth and development, and optimal hydrothermal conditions occurred only in April 2015 and September 2016.

The concentration of NDF and ADF in biomass was also dependent on the harvest, which was confirmed by similar studies conducted by Särkijärvi et al.[37].

An important element of the nutritional value of roughage is its digestibility. It depends on many factors such as harvest time or weather conditions[38, 39, 40, 41]. Roughage digestibility is dependent on the content of NDF and ADF[36]. *Phleum pratense* biomass from the present study was generally of good digestibility. However, for plants treated with slurry applied together with vermicompost extract or leonardite extract or with NPK mineral fertilizers, it was significantly lower than for control plants. For plants treated with slurry on its own, digestibility was significantly lower only in relation to *Phleum pratense* treated with slurry supplemented with vermicompost extract. Differentiation of forage grass digestibility in response to soil conditioners was also found in the research by Truba et al.[20] and Baert and VanWaes[42]. Across the harvests, the lowest digestibility was recorded in the third one, which is not confirmed by the research of Truba et al.[20]. In many other studies, its value decreased in consecutive harvests[43, 44]. In research on grass-legume mixtures harvested five times, Gaweł[45] recorded digestibility of 80%, which means that it depends not only on the harvest but also on the frequency of mowing.

The content of fibrous fractions, which increases with plant aging, translates into dry matter intake (DMI)[41]. In the present research, supplementing slurry with soil conditioners or NPK fertilizers significantly reduced DMI values compared to plants treated with slurry applied on its own and to control plants. DMI value recorded for hay by Moore and Undersander[46] ranged from 1.93 to 2.17%. Values for *Dactylis glomerata* and *Lolium perenne* treated with similar extracts recorded by Truba et al.[20] were similar to the results of the present research.

The highest DMI was found for the first harvest, significantly higher than the value for the third one, which was confirmed by the research of Truba et al.[20]. Considering years of research, significantly higher DMI values were recorded for biomass obtained in the first and second (2015 and 2016) than in the third (2017). The decrease in the DMI value in the third year of research was a result of unfavorable weather conditions across this growing period.

The relative feed value (RFV) of *Phleum pratense* biomass on slurry treated plots supplemented with both extracts or NPK minerals was significantly lower than the RFV of control plants and those treated with slurry on its own. The value calculated according to the Linn and Martin test[11] [1989] made it possible to qualify forage from the control plot and from the plot treated only with slurry to quality class I, meeting the nutritional requirements of high-yielding dairy cows. Forage from plots where slurry was supplemented with soil conditioners or NPK fertilizers was qualified to quality class II, useful for feeding good dairy cows and young heifers selected for breeding. RFV of plants from those plots was lower by approx. 8.0–11.0% than of biomass treated with slurry alone.

Conclusions

Phleum pratense from plots with slurry supplemented with soil conditioners or NPK fertilizers significantly differed in NDF and ADF content compared to plants fertilized only with slurry, with the exception of ADF content in plants treated with slurry supplemented with leonardite extract. Dry matter intake and digestibility (DMI and DDM) for biomass treated with slurry alone were significantly lower than when treated with slurry applied together vermicompost extract or with NPK fertilizers, which translated into an improvement in the relative feed value (RFV).   The data confirmed the research hypothesis because supplementing slurry with vermicompost extract or NPK fertilizers improved the parameters more than slurry applied on its own.

The values of *Phleum pretense* parameters in response to slurry supplemented with NPK mineral fertilizers differed significantly from those recorded for plants treated with slurry supplemented with vermicompost extract or leonardite extract. The content of NDF and ADF and the values of DMI, DDM and RFV indicated that the application of slurry together with both extracts did not improve the nutritional quality of *Phleum pratense* forage more than slurry supplemented with NPK minerals. The relative feed value of biomass collected from the slurry and NPK fertilizer plot was higher than the value of plants treated with slurry supplemented with the leonardite extract, but comparable to the value of biomass treated with slurry supplemented with vermicompost extract.

Declarations

Data availability

The datasets generated and analysed during the current study are not publicly available due to they are the authors' own data, but are available from the corresponding author on reasonable request.

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Author contributions

Conceived the experiment: B.W-K.

Project planning: B.W-K.

Experimental work: B.W-K.

Resources: B.W-K. and E.M.

Data analysis: B.W-K.

Prepared tables: B.W-K. and E.M.

Wrote the main manuscript text: B.W-K.

Both authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

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