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Changes in Selected Forage Parameters of Meadow Timothy (*Phleum pratense* L.) Biomass and in the Soil as a Response to Slurry Interaction with Soil Conditioners

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Abstract: The aim of the research was to assess the effect of slurry and soil conditioners on *Phleum pratense* yield and its selected parameters and on soil nutrients. The second aspect of the experiment was to obtain information whether slurry and soil conditioners applied together will increase the accumulation of nitrogen, phosphorus and potassium in the soil compared to slurry applied on its own or together with NPK fertilizers. The research was carried out on the basis of a three-year (2015–2017) field experiment. The interaction of soil conditioners or mineral fertilizers with slurry was tested on forage grass of the Meadow timothy species of the Secesja variety, sown in autumn 2014. Grass total protein, crude fiber and crude ash content and net energy concentration (NE) were determined; additionally, at the end of the experiment, total nitrogen, phosphorus and potassium content in the soil was measured. The results of plant material analyses were statistically developed using a three-factor analysis of variance, while the results of soil content determination were developed with a one-factor analysis. The interaction of slurry with Humus Active or Rosahumus significantly increased the amount of meadow timothy biomass in relation to plants fertilized with slurry on its own (by 22% and 26%, respectively) or supplemented with NPK fertilizers (by 9% and 7%). The interaction of slurry with the Rosahumus soil conditioner resulted in a significant increase in meadow timothy crude fiber content compared to the effect of slurry applied on its own or with NPK fertilizers (by 5% and 6.5%). The interaction of slurry with soil conditioners did not result in a significant increase in total protein and crude ash content or in the net energy concentration compared to slurry applied on its own or with NPK fertilizers. Soil nutrient content determined after the experiment proved a significant impact of the interaction of soil conditioners and slurry on the accumulation of nitrogen, phosphorus and potassium.

Keywords: bovine slurry; soil conditioners; forage grass; forage value; soil nutrients

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1. Introduction

Agriculture, animal husbandry in particular, is a significant source of environmental pollution. Slurry, a liquid by-product of animal production, is a very valuable fast-acting fertilizer with a very high concentration of macro and micronutrients, mainly in absorbable forms. However, its improper storage and use may contribute to the contamination of the environment [1]. It is predicted that both in the countries of the European Union and Eastern Europe, livestock production will develop, and thus ecological problems related to the management of slurry will also increase [2].

The rules for the storage and use of slurry in European Union countries are regulated by the Nitrate Directive and the Code of Good Agricultural Practice [3,4], which sets 170 kg·ha^{-1} as a maximum annual dose of total nitrogen. With a decreasing area of arable fields and a rising stocking density per hectare of agricultural land, meeting these conditions is often impossible [2,5].

Nutrients in slurry are in forms more easily absorbed by plants than in manure, which is why it is a fast-acting fertilizer [6]. In slurry, apart from organic forms, nitrogen is present in ammonia, ammonium compounds, nitrates and nitrogen oxides [7]. According to Christensen et al. [8], nitrogen in ammonium compounds can constitute up to 70% of its total amount, and if it is not taken up by plants, it is easily lost.

Studies on the application of slurry to grassland have demonstrated its beneficial effect on the yield and forage quality [9–11], but also on soil physicochemical [12] and biological properties [13]. Recently, some attempts have been made to find ways of mitigating the negative impact of slurry on the environment by reducing ammonia (NH_3) emission [14] or intensifying carbon sequestration [15].

Soil fertility can be increased not only by organic fertilizers. Another way is to use soil conditioners, which, according to the Act on Fertilizers and Fertilization [16] Article 2(1)(7), are "substances added to the soil in order to improve its properties or its chemical, physical, physicochemical or biological parameters". These substances are known as soil conditioners, biological fertilizers and biostimulants [17]. Products that improve soil fertility, and thus increase the yield and quality of crops, include Rosahumus and Humus Active.

The Rosahumus soil conditioner is produced from leonardite, an oxidized form of lignite, while Humus Active is produced on the basis of vermicompost. Humus Active improves soil properties, increasing, among others, use efficiency of nutrients from mineral and organic fertilizers and from organically bound soil macro and microelements, which increases plant yield and its quality. Rosahumus is an organomineral product improving soil fertility with its nutrient concentration 12 times greater than soil mineral fraction. Its organic compounds have high water capacity, and they bind insoluble metal ions, oxides and hydroxides, releasing them slowly. Those organic compounds increase the conversion of macro and micronutrients to available forms, and also act as a natural agent chelating metal ions.

Thanks to these properties, soil conditioners are used mainly by organic farmers, but more and more often by traditional ones to treat vegetables, field crops and ornamental plants, as well as orchards and grassland [18–24].

Meadow timothy is a grass species grown on permanent grassland as well as on arable land. It requires fertile soil and is characterized by winter hardiness and frost resistance, but it is drought susceptible due to a shallow root system. It is both palatable and nutritional, with a high content of water-soluble carbohydrates [25–27]. However, due to a large proportion of generative shoots containing significant amounts of fiber, its digestibility is reduced. According to studies carried out by Bélanger et al. [28] and Thorvaldson et al. [29,30], the dry matter yield of this species is negatively correlated with its nutritional value, which indicates that an increase in the yield may affect its quality.

The aim of the study was to determine the effect of the interaction of slurry with the Rosahumus and Humus Active soil conditioners on the yield and selected parameters of meadow timothy nutritional value and on the total content of nitrogen, potassium and phosphorus in the soil.

2. Materials and Methods

2.1. Experimental Conditions

The research was conducted as a three-year (2015–2017) field experiment located at the University of Natural Sciences and Humanities' experimental plot in Siedlce, Poland (52°10′ N, 22°17′ E), in three replications in a random block layout and on plots of 4.5 m^2. The experiment was established on soil with a granulometric composition of loamy sand, included in the order of anthropogenic soils, culture-earth type and hortisole subtype [31]. Its organic carbon concentration was 14.50 $g \cdot kg^{-1}$ DM, with a total nitrogen concentration of 1.30 $g \cdot kg^{-1}$ DM, the C:N ratio of 11.0:1 and slightly acidic pH of 6.5. The amounts of absorbable forms of phosphorus (P_2O_5 = 13.7 $mg \cdot 100^{-1}$ g of the soil), potassium

(K_2O = 14.5 mg·100^{-1} g of the soil) and magnesium (Mg = 6.5 mg·100^{-1} g of the soil) corresponded to moderate content.

2.2. Treatment Scheme

The fertilizer used in the experiment was slurry from dairy cows, used separately and supplemented with NPK fertilizers or soil conditioners, with the trade names of Rosahumus and Humus Active. Thus, the experiment consisted of the following research units:

(a) control (without fertilizers);
(b) slurry;
(c) slurry + Rosahumus;
(d) slurry + Humus Active;
(e) slurry + NPK fertilizers.

Bovine slurry was applied each year at a total dose of 30 $m^3 \cdot ha^{-1}$, divided into three equal parts applied before each growth cycle. Its C:N ratio (8:1) was narrow, with a dry matter content of 10%, and a content of N = 31.0; P = 7.10; K = 32.5 g·kg^{-1} DM.

In the experiment, the soil conditioners, agents improving soil properties, were used according to the guidance of the Institute of Cultivation, Fertilization and Soil Science in Puławy [17]. Rosahumus, produced by Agrosimex Ltd. (Goliany, Poland) is an organo-mineral fertilizer with 56% of organic matter, including 85% of humic acids, about 100 g·kg^{-1} of potassium and about 5 g·kg^{-1} of iron. The Humus Active soil conditioner produced by Ekodarpol—Commercial and Trade Company (Dębno, Poland) contains billions of beneficial microorganisms, including phosphate-solubilizing and cellulolytic bacteria, azotobacter and actinomycetes. It also contains 55% saturated humic acids, including 49% humin acids, 1% fulvic acids and 5% humins and ulmins. The product contains nitrogen (0.2 g·kg^{-1}), phosphorus (1.3 g·kg^{-1}), potassium (3.0 g·kg^{-1}) and microelements with 0.45 g·kg^{-1} of iron.

Soil conditioners were applied annually, at the beginning of the growing period, in doses recommended by the manufacturer, i.e., Rosahumus at 3 kg·ha^{-1} and Humus Active at 20 L·ha^{-1}. Mineral fertilizer doses were as follows: N = 100 kg·ha^{-1} in the form of ammonium nitrate (NH_4NO_3); P (P_2O_5) = 80 kg·ha^{-1} in the form of triple superphosphate ($H_2(PO_4)_2$); K (K_2O) = 120 kg·ha^{-1} in the form of potassium chloride (KCl). Phosphorus was applied once at the beginning of the growing period (the last week of March), while nitrogen and potassium were applied in three equal doses: the first before the start of the growing period (the last week of March), the second after the first harvest (the first week of June) and the third after the second harvest (the third week of July). The interaction of soil conditioners and mineral fertilizers with slurry was studied using meadow timothy, a forage grass species of the Secesja variety, sown in autumn 2014 in accordance with the seeding standard.

2.3. Sample Preparation, Chemical and Statistical Analyses

During three years of its full use, grass was harvested three times a year, and samples of soil material were collected at the end of each growing period. Immediately after the harvest, green matter from each fertilizer plot was weighed and 0.5 kg samples were dried naturally in a ventilated room. The air-dried samples were weighed to determine dry matter content with the drying-weighing method. Then, the dry grass was chaffed and a homogeneous sample was collected and crushed. Samples of soil material from each fertilizer plot were air-dried and sieved through a mesh of 2 mm.

In the plant material prepared in this way, total protein, ash and crude fiber content was determined by near-infrared reflectance spectroscopy (NIRS) using the LSDFlex N-500 apparatus, with INGOT ready-made calibrations for dry forage. The method is described in detail in the Polish Standard PN-EN ISO 120099:2010 and in the literature [32,33].

The net energy concentration (NE) was calculated using the formula based on crude fiber content [34]:

$$NE = 1.50 - 0.02 \times CF \quad (1)$$

where:

NE—net energy concentration (ou·kg^{-1} DM);

CF—crude fiber content in % DM.

Total nitrogen content in the soil was determined by the Kjeldahl method at the end of the experiment (in the third year of the experiment), after wet mineralization of the samples in concentrated sulfuric acid, with distillation from a strongly alkaline medium and titration with a solution of sulfuric acid with a strictly defined titer. The total content of phosphorus and potassium was determined by the ICP method, after mineralization of the soil material at a temperature of 450 °C. Ash formed after mineralization was treated with hydrochloric acid solution (HCl:H_2O = 1:1) to dissolve carbonates and extract silica. In the chloride-containing solution thus prepared, the total content of phosphorus and potassium was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES).

The results of plant material analysis were statistically processed using the analysis of variance for a three-factor experiment [35]. The following research variables were considered: (A)—treatment (5 levels); (B)—growing periods (3 levels); (C)—grass growth cycles (3 levels). One factor analysis of variance for experimental replicates did not show any significant differences between them.

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

where:

y_{ijlp}—the value of the variable for the i-th level of factor A and p-th level of factor C for the j-th replicate;

m—the mean of research;

a_i, b_l, c_p—the effects of factors;

g_j—the effect of the j-th replicate;

ab_{il}, ac_{ip}, bc_{lp}—the effects of the interaction of two factors;

abc_{ilp}—the effect of the interaction of three factors;

$e^{/1/}{}_{ij}$, $e^{/2/}{}_{ijlp}$—the effect of random factor;

i = 1, 2, . . . , a; a—the number of levels of factor A;

j = 1, 2, . . . , n; n—the number of replicates;

l = 1, 2, . . . , b; b—the number of levels of factor B;

p = 1, 2, . . . , c; c—the number of levels of factor C.

The results of soil material analysis were statistically processed using variance analysis for univariate experiments according to the following mathematical model:

$$y_{ij} = m + a_i + e_{ij} i = 1, 2, \ldots, a \\ j = 1, 2, \ldots, n_i \quad (3)$$

where:

m—the total mean;

a_i—effect of group number i;

e_{ij}—random error effect.

The significance of differences between means was estimated with Tukey's test at the significance level $\alpha \leq 0.05$. Statistica 6.0 was used for the calculations [36].

2.4. Weather Conditions

In order to determine the temporal variability of weather conditions (Table 1) and their impact on plant growth, Sielianinov's hydrothermal coefficient [37] was determined on the basis of monthly precipitation (P) and the monthly sum of mean daily temperatures (Σt) using the formula [38]:

$$K = P/0.1\ \Sigma t \quad (4)$$

Table 1. Selianinov's hydrothermal coefficient throughout the growing period.

Year	Month						
	April	May	June	July	August	September	October
2015	1.36 (o) *	1.87 (mw)	1.64 (mw)	0.59 (sd)	1.92 (mw)	0.64 (sd)	0.12 (ed)
2016	1.22 (md)	2.63 (sw)	0.87 (d)	1.08 (md)	0.18 (ed)	1.46 (o)	1.94 (mw)
2017	2.88 (sw)	1.15 (md)	1.08 (md)	0.45 (sd)	0.96 (d)	1.92 (mw)	1.90 (mw)

* Values are rated as: $K \leq 0.4$ extremely dry (ed), $0.4 < K \leq 0.7$ severely dry (sd), $0.7 < K \leq 1.0$ dry (d), $1.0 < K \leq 1.3$ moderately dry (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).

During the three years of research, optimal hydrothermal conditions were only in April 2015 and September 2016. In the second and third growing periods (2016 and 2017), quite dry, dry and very dry months definitely prevailed.

3. Results

3.1. Yield

The yield of meadow timothy (Table 2) varied significantly depending on the treatment and harvest. The most biomass (the average value for the experiment) was produced on the plot treated with both slurry and Rosahumus (13.70 Mg·ha^{-1}), while the least (10.08 Mg·ha^{-1}) on the plot where only slurry was used.

Table 2. The effect of treatment, growing period and harvest on meadow timothy yield (Mg·ha^{-1} DM).

Growing period (B)	Harvest (C)	Treatment (A)					Mean (±SD) ****
		O *	S	S + HA	S + R	S + NPK	
2015	I	3.20	5.70	6.50	7.30	5.80	5.70 (1.54)
	II	2.00	3.50	3.80	4.20	3.90	3.48 (0.86)
	III	1.45	1.33	2.60	2.00	1.95	1.87 (0.50)
	Total	6.65	10.53	12.90	13.50	11.65	11.05
2016	I	2.70	5.10	7.20	6.20	4.80	5.20 (1.70)
	II	2.40	3.60	4.10	4.60	4.20	3.78 (0.85)
	III	0.85	1.80	2.40	3.70	3.40	2.43 (1.16)
	Total	5.95	10.50	13.70	14.50	12.40	11.41
2017	I	2.30	4.50	5.50	5.20	6.20	4.74 (1.70)
	II	2.10	3.10	3.80	4.80	3.70	3.50 (1.00)
	III	1.30	1.60	3.00	3.10	1.40	2.08 (0.90)
	Total	5.70	9.20	12.30	13.10	11.30	10.32
Mean across growing periods (±SD)		6.10 d ** (0.49)	10.08 c (0.76)	12.97 a (0.70)	13.70 a (0.72)	11.80 b (0.56)	10.92 (0.55)
Mean across harvests (±SD)							
	I	2.73 (0.45)	5.10 (0.60)	6.40 (0.85)	6.23 (1.05)	5.60 (0.72)	5.21 A *** (0.48)
	II	2.17 (0.21)	3.40 (0.26)	3.90 (0.17)	4.53 (0.30)	3.93 (0.25)	3.59 B (0.17)
	III	1.20 (0.31)	1.58 (0.23)	2.67 (0.30)	2.93 (0.86)	2.25 (1.00)	2.12 C (0.28)

* O—control (without fertilizers), S—slurry, S + HA—slurry + Humus Active, S + R—slurry + Rosahumus, S + NPK—slurry + NPK fertilizers; ** The means in the lines marked with small different letters a, b, c, d (for treatment) differ significantly at $p \leq 0.05$; *** The means in the columns marked with different capital letters A, B, C (for the harvest) differ significantly at $p \leq 0.05$; **** (±SD)—standard deviation.

The Rosahumus soil conditioner applied together with slurry increased the yield of grass by 26% in relation to plants treated with slurry only. In comparison with slurry used in combination with NPK fertilizers, complementary treatment with slurry and soil conditioners also resulted in a 9% increase for Humus Active and a 7% increase for Rosahumus.

The amounts of biomass collected from the plot treated with slurry and Humus Active on the one hand and from the plot with slurry and Rosahumus on the other were not significantly different. The annual yields of meadow timothy across growing periods were very similar and ranged from 11.40 Mg·ha^{-1} in the second year to 10.32 Mg·ha^{-1} in the third. Across harvests, the most biomass, the average of treatments and growing periods, was obtained in the first one (5.21 Mg·ha^{-1}), while the least (2.12 Mg·ha^{-1}) in the third.

3.2. Total Protein Content

Total protein content (Table 3) significantly varied across treatments, growing periods and harvests. The highest value (142.4 g·kg^{-1}) was recorded on the plot with slurry applied together with Rosahumus. The protein content of grass from this plot was only 1.5% higher than that of plants treated with slurry and about 4% higher than that of those treated with slurry supplemented with NPK fertilizers. Compared to other treatments, the significantly lowest total protein content (133.0 g·kg^{-1}) was found in meadow timothy treated with slurry applied together with the Humus Active soil conditioner. Slurry applied together with Rosahumus or NPK did not significantly increase the amount of protein compared to biomass fertilized with slurry alone. Biomass collected in the first growing period contained the most total protein (140.6 g·kg^{-1}), while a 4% lower amount (134.9 g·kg^{-1}) was recorded in the third year. Across harvests (as the average of treatments and growing seasons), the most total protein (141.9 g·kg^{-1}) was in the first, but in the third it was 10% lower (134.9 g·kg^{-1}).

Table 3. The effect of treatment, growing period and harvest on meadow timothy protein content (g·kg^{-1} DM).

Growing Period (B)	Harvest (C)	Treatment (A)					Mean (±SD) ****
		O *	S	S + HA	S + R	S + NPK	
2015	I	135.1	156.7	140.2	153.1	155.6	148.1 (9.8)
	II	123.4	149.3	135.9	145.0	153.4	141.4 (11.9)
	III	113.4	135.5	135.4	136.5	140.0	132.2 (10.6)
	Mean (±SD)	123.9 (10.9)	147.2 (10.7)	137.2 (2.6)	144.9 (8.3)	149.7 (8.4)	140.6 A *** (3.3)
2016	I	121.8	157.6	136.7	153.8	139.2	141.8 (14.4)
	II	113.9	140.0	130.0	150.0	135.5	133.9 (13.4)
	III	125.6	142.5	125.5	138.4	120.9	130.6 (9.3)
	Mean (±SD)	120.4 (5.9)	146.7 (9.5)	130.7 (5.6)	147.4 (8.0)	131.9 (9.7)	135.4 AB (1.9)
2017	I	126.9	138.0	140.8	139.5	134.8	136.0 (5.5)
	II	120.4	123.5	132.8	135.0	128.3	128.0 (6.1)
	III	116.8	118.9	120.0	130.0	123.3	121.8 (5.5)
	Mean (±SD)	121.4 (5.1)	126.8 (10.0)	131.2 (10.5)	134.8 (4.7)	128.8 (5.8)	128.6 B (2.8)
Mean across growing periods (±SD)		121.9 d ** (1.8)	140.2 a (11.6)	133.0 c (3.6)	142.4 a (6.6)	136.8 a (11.3)	134.9 (4.4)
Mean across harvests (±SD)							
	I	127.9 (6.7)	150.8 (11.1)	139.2 (2.2)	148.8 (8.1)	143.2 (11.0)	141.9 (3.6)
	II	119.2 (4.8)	137.6 (13.1)	132.9 (2.9)	143.3 (7.6)	139.1 (13.0)	134.4 (4.6)
	III	118.6 (6.3)	132.3 (12.1)	126.9 (7.8)	134.9 (4.4)	128.1 (10.4)	128.2 (3.1)

* O—control (without fertilizers), S—slurry, S + HA—slurry + Humus Active, S + R—slurry + Rosahumus, S + NPK—slurry + NPK fertilizers; ** The means in the lines marked with small different letters a, c, d (for treatment) differ significantly at $p \leq 0.05$; *** The means in the columns marked with different capital letters A, B (for the growing period) differ significantly at $p \leq 0.05$; **** (±SD)—standard deviation.

3.3. Crude Ash

The content of crude ash (Table 4) was significantly affected only by treatment. The values from plots treated with slurry supplemented with Humus Active (111.3 g·kg^{-1}), or with Rosahumus (111.0 g·kg^{-1}), or with NPK fertilizers (112.8 g·kg^{-1}) were very similar.

Crude ash content was relatively low in grass treated only with slurry (104.4 g·kg^{-1}), but it was not significantly lower than on plots where slurry was supplemented with soil conditioners or with mineral fertilizers. The use of slurry with soil conditioners and NPK fertilizers significantly increased the content of crude ash (by 12–13%) only in relation to the control plot (without fertilizers). Crude ash content across growing periods (as the average value across treatments) practically did not vary and was not affected by thermal and humidity conditions. Of all harvests, it was 2% higher in plants of the second one than in those of the first or third.

Table 4. The effect of treatment, growing period and harvest on meadow timothy crude ash content (g ·kg^{-1} DM).

Growing Period (B)	Harvest (C)	Treatment (A)					Mean (±SD) ****
		O *	S	S + HA	S + R	S + NPK	
2015	I	96.7	106.8	107.9	109.0	115.3	107.1 (6.7)
	II	85.5	114.5	113.5	115.3	107.8	107.3 (12.5)
	III	103.7	100.3	120.7	108.0	110.0	108.5 (7.8)
	Mean (±SD)	95.3 (9.2)	107.2 (7.1)	114.0 (6.4)	110.8(3.9)	111.0 (3.8)	107.7 (3.1)
2016	I	84.0	96.7	114.9	112.7	114.3	104.5 (13.7)
	II	97.8	105.3	108.7	114.3	120.7	109.4 (8.7)
	III	101.7	111.2	105.3	103.5	115.9	107.5 (5.9)
	Mean (±SD)	94.5 (9.3)	104.4 (7.3)	109.6 (4.9)	110.2 (5.8)	116.9 (3.3)	107.1 (4.0)
2017	I	106.3	88.7	108.9	113.6	120.3	107.6 (11.8)
	II	110.5	103.5	114.3	117.2	108.0	110.7 (5.3)
	III	94.8	112.7	107.5	105.4	103.0	104.7 (6.6)
	Mean (±SD)	103.9 (8.1)	101.6 (12.1)	110.2 (3.6)	112.1 (6.0)	110.4 (8.9)	107.6 (3.4)
Mean across growing periods (±SD)		97.9 b ** (5.2)	104.4 ab (2.8)	111.3 a (2.4)	111.0 a (1.0)	112.8 a (3.6)	107.5 (1.0)
Mean across harvests (±SD)							
	I	95.7 (11.2)	97.4 (9.1)	110.6 (3.8)	111.8 (2.4)	116.6 (3.2)	106.4 (1.6)
	II	97.9 (12.5)	107.8 (5.9)	112.2 (3.0)	115.6 (1.5)	112.2 (7.4)	109.1 (1.7)
	III	100.1 (4.7)	108.1 (6.8)	111.2 (8.3)	105.6 (2.2)	109.6 (6.5)	106.9 (2.0)

* O—control (without fertilizers), S—slurry, S + HA—slurry + Humus Active, S + R—slurry + Rosahumus, S + NPK—slurry + NPK fertilizers; ** The means in the lines marked with small different letters a, b (for treatment, interaction of treatment and the growing period) differ significantly at $p \leq 0.05$; **** (±SD)—standard deviation.

3.4. Crude Fiber

The crude fiber content (Table 5) of meadow timothy varied significantly across treatments, growing periods and harvests. As the average value for the experiment, its largest amount (302.3 g·kg^{-1} DM) was found in the grass treated with slurry applied together with Rosahumus. It was the lowest in control plants and in those treated with slurry combined with NPK fertilizers (283.9 g·kg^{-1} DM). The fiber content of plants treated with slurry applied together with Humus Active and with slurry applied with Rosahumus did not differ significantly. The average crude fiber content of the biomass varied across the experimental years. The highest value was found (303.1 g·kg^{-1} DM) in the third (2017) year of research, with 9% less (274.8 g·kg^{-1} DM) in the first (2015). Across harvests (the average of treatments and years of research), the most crude fiber (301.1 g·kg^{-1} DM) was in the third, but in the first it was 6% less (282.8 g·kg^{-1} DM).

3.5. Net Energy (NE)

The concentration of net energy (Table 6) in meadow timothy varied significantly depending on the treatment, year of research and harvest. The highest (the average of growing periods) concentration of net energy in the forage was found on the plot where slurry and NPK fertilizers (0.94 ou·kg^{-1} DM) were used, while the lowest in plants from the plot on which the Rosahumus (0.90 ou·kg^{-1} DM) soil conditioner was applied together with slurry. In relation to the plot treated both with slurry and Rosahumus, a significantly higher net energy concentration was recorded on the plots treated only with slurry and with slurry supplemented with NPK fertilizers.

The highest net energy concentration (the average of treatments and harvests) in meadow timothy biomass (0.95 ou·kg^{-1} DM) was recorded in the first year (2015), while the lowest in the third (0.90 ou·kg^{-1} DM). Across harvests (the average of treatments and growing periods), the highest concentration of net energy (0.95 ou·kg^{-1} DM) was recorded for the first harvest forage and the smallest (0.90 ou·kg^{-1} DM) for the third.

3.6. Nitrogen, Phosphorus and Potassium in Soil

Determined at the end of the experiment, the content of nitrogen, phosphorus and potassium in the soil (Table 7) significantly varied depending on the treatment. Significantly the highest amount of total nitrogen in the soil (1.69 g·kg^{-1} DM) was on the plot treated with slurry and Humus Active, the lowest (1.37 g·kg^{-1} DM) on the plot where only slurry was used. Additionally, combined application of NPK fertilizers and slurry resulted in a significant nitrogen content increase in the soil, compared to the plot with slurry only. In the soil from the plot treated both with slurry and Rosahumus, significantly more phosphorus was recorded (0.85 g·kg^{-1} DM) than in the soil fertilized with slurry only (0.62 g·kg^{-1} DM), or with slurry supplemented with Humus Active (0.58 g·kg^{-1} DM).

Table 5. The effect of treatment, growing period and harvest on meadow timothy crude fiber content (g·kg^{-1} DM).

Growing Period (B)	Harvest (C)	Treatment (A)					Means (±SD) ****
		O *	S	S + HA	S + R	S + NPK	
2015	I	254.6	269.4	270.5	267.4	276.4	267.7 (8.0)
	II	260.5	250.5	285.3	290.7	290.4	275.4 (18.7)
	III	280.7	260.8	280.7	300.8	283.7	281.3 (14.2)
	Mean (±SD)	265.3 ab ** (13.7)	260.2 b (9.5)	278.8 a (7.6)	286.3 a (17.1)	283.5 a (7.0)	274.8 C *** (5.5)
2016	I	285.3	279.5	289.7	299.7	274.6	285.8 (9.7)
	II	290.4	286.3	321.5	300.4	265.9	292.9 (20.3)
	III	312.8	305.6	330.5	316.5	280.7	309.2 (15.2)
	Mean (±SD)	296.2 ab (14.6)	290.5 bc (13.5)	313.9 a (21.4)	305.5 ab (9.5)	273.7c (7.4)	295.9 B (5.7)
2017	I	286.7	297.4	289.6	315.6	286.3	295.1 (12.3)
	II	294.2	305.6	290.4	298.4	290.6	295.8 (6.3)
	III	290.4	314.5	320.8	330.8	307.3	312.7 (15.2)
	Mean (±SD)	290.4 b (3.7)	305.8 ab (8.5)	300.3 a (17.8)	314.9 a (16.2)	294.7 ab (11.1)	301.3 A (4.5)
Means across growing periods (±SD)		283.9 b (16.4)	285.5 b (23.2)	297.7 ab (17.7)	302.3 a (14.6)	283.9 b (10.5)	290.7 (13.9)
Mean across harvests (±SD)							
	I	275.5 (18.1)	282.1 (14.2)	283.3 (11.0)	294.2 (24.5)	279.1 (6.3)	282.8 C (13.9)
	II	281.7 (18.4)	280.8 (27.9)	299.1 (19.6)	296.5 (5.1)	282.3 (14.2)	288.1 B (11.0)
	III	294.6 (16.5)	293.6 (28.8)	310.7 (26.4)	316.0 (15.0)	290.6 (14.6)	301.1 A (17.2)

* O—control (without fertilizers), S—slurry, S + HA—slurry + Humus Active, S + R—slurry + Rosahumus, S + NPK—slurry + NPK fertilizers; ** The means in the lines marked with small different letters a, b, c (for treatment, interaction of treatment and the growing period) differ significantly at $p \leq 0.05$; *** The means in the columns marked with different capital letters A, B, C (for the growing period and for the harvest) differ significantly at $p \leq 0.05$, **** (±SD)—standard deviation.

Table 6. The effect of treatment, growing period and harvest on meadow timothy net energy concentration (NE) (ou·kg^{-1} DM).

Growing Period (B)	Harvest (C)	Treatment (A)					Mean (±SD) ****
		O *	S	S + HA	S + R	S + NPK	
2015	I	0.98	0.96	0.96	0.96	0.96	0.96 (0.02)
	II	0.97	1.00	0.93	0.92	0.92	0.95 (0.04)
	III	0.94	0.98	0.94	0.89	0.93	0.94 (0.03)
	Mean (±SD)	0.97 ab (0.03)	0.98 a (0.02)	0.94 ab (0.01)	0.93 b (0.03)	0.93 b (0.01)	0.95 A (0.01)
2016	I	0.93	0.94	0.92	0.90	0.95	0.93 (0.02)
	II	0.92	0.93	0.86	0.90	0.97	0.91 (0.01)
	III	0.87	0.89	0.84	0.87	0.94	0.88 (0.01)
	Mean (±SD)	0.90 bc (0.03)	0.92 ab (0.03)	0.87 c (0.04)	0.89 c (0.02)	0.95 a (0.01)	0.91 B (0.01)
2017	I	0.92	0.90	0.92	0.87	0.93	0.91 (0.02)
	II	0.91	0.89	0.92	0.90	0.92	0.91 (0.01)
	III	0.92	0.87	0.86	0.84	0.88	0.88 (0.03)
	Mean (±SD)	0.90 ab (0.01)	0.89 ab (0.02)	0.90 ab (0.03)	0.87 b (0.03)	0.92 a (0.02)	0.90 B (0.01)
Mean across growing periods (±SD)		0.91 ab (0.03)	0.93 a (0.04)	0.91 ab (0.03)	0.90 b (0.03)	0.94 a (0.02)	0.91 (0.03)
Mean across harvests (±SD)							
	I	0.95 (0.04)	0.93 (0.03)	0.93 (0.02)	0.91 (0.05)	0.94 (0.01)	0.93 (0.03)
	II	0.94 (0.04)	0.94 (0.06)	0.90 (0.04)	0.91 (0.01)	0.93 (0.03)	0.92 (0.02)
	III	0.91 (0.03)	0.91 (0.06)	0.88 (0.05)	0.87 (0.03)	0.92 (0.03)	0.90 (0.03)

* O—control (without fertilizers), S—slurry, S + HA—slurry + Humus Active, S + R—slurry + Rosahumus, S + NPK—slurry + NPK fertilizers; The means in the lines marked with small different letters a, b, c (for treatment, interaction of treatment and the growing period) differ significantly at $p \leq 0.05$; The means in the columns marked with different capital letters A, B (for the growing period) differ significantly at $p \leq 0.05$; **** (±SD)—standard deviation.

Table 7. The effect of treatment, growing period and harvest on soil NPK accumulation (g·kg^{-1} DM).

Treatment	Macronutrients (g·kg^{-1} DM)		
	N	P	K
O *	1.08 d **	0.63 b	1.25 b
S	1.37 c	0.62 b	1.40 b
S + HA	1.69 a	0.58 b	1.68 a
S + R	1.46 abc	0.85 a	1.74 a
S + NPK	1.61 ab	0.74 ab	1.80 a
Mean (±SD) ***	1.44 (0.24)	0.67 (0.11)	1.58 (0.25)

* O—control (without fertilizers), S—slurry, S + HA—slurry + Humus Active, S + R—slurry + Rosahumus, S + NPK—slurry + NPK fertilizers; ** The means in the columns marked with different small letters a, b, c, d (for the growing period) differ significantly at $p \leq 0.05$; *** (±SD)—standard deviation.

Recorded at the end of the experiment, the highest amount of potassium in the soil (1.80 g·kg^{-1} DM) was on plots treated with slurry combined with NPK fertilizers, the lowest (1.46 g·kg^{-1} DM) in the soil from plots where slurry was used on its own. Slurry used together with either soil conditioner or with NPK fertilizer significantly increased the accumulation of potassium in the soil compared to plots with slurry only. Potassium content in the soil treated with slurry applied with NPK fertilizers did not differ significantly from the effect of slurry applied together with either soil conditioner.

4. Discussion

The need for more accurate balancing of livestock rations and increasing demands for good quality grassland forage require the most accurate determination of the latter's nutritional value [39,40]. The yield and quality of forage grasses are determined by a number of factors, such as treatment, weather conditions or growth cycle. According to Sikorra and Zimmer-Gajewska [41] and Kulik [42], meadow timothy is a high-quality species of alternating grassland, short-lived but producing high yields.

Compared to the effects of slurry applied on its own, its interaction with the Humus Active or Rosahumus soil conditioner resulted in a significant increase in the meadow timothy yield (by 22% and 26%). Kryszak et al. [43] found that NPK fertilizers determined the yield of forage grasses to the greatest extent. In their experiment, the yields of meadow timothy were at a similar level to those of the present experiment. In turn, using slurry and the Humus Active and UGmax soil conditioners, Wiśniewska-Kadżajan and Jankowski [18,19] noted that the resulting yields of *Festulolium braunii* and *Lolium multiflorum* were not significantly different compared to plants treated exclusively with slurry. A clear increase in the amount of meadow timothy biomass as an effect of the interaction between slurry and soil conditioners without a strong response to complementary NPK fertilizer treatment is probably related to the fact that it is not a nitrogen-loving species.

Compared to the effect of slurry applied on its own or together with NPK fertilizers, no significant effect of the interaction of slurry and soil conditioners on total protein content was observed. According to Grygierzec [44], optimal total protein content in forage, necessary for a proper digestion process, should range from 150.0 g·kg^{-1} DM to 170.0 g·kg^{-1} DM. However, it does not always increase in proportion to the amount of nitrogen applied to plants. This may be due to protein dilution in the higher yield [45–48].

Total protein content of timothy treated with slurry applied together with Rosahumus did not increase significantly compared to the effect of slurry applied on its own, in contrast to the combined use of slurry and Humus Active, which resulted in its decrease. Although Rosahumus did not introduce nitrogen into the soil, when combined with slurry it resulted in protein content similar to that of NPK fertilizers applied with slurry. This was probably due to the ability of the Rosahumus conditioner to increase the conversion of nutrients, especially of nitrogen, into forms available to plants.

According to Nazaruk et al. [49], crude ash content exceeding 150.0 g·kg^{-1} DM indicates contamination of plant material with soil. In the present studies, it ranged from 97 g·kg^{-1} DM in control plants to 112.8 g·kg^{-1} DM in those treated with slurry and NPK fertilizers. That indicates that biomass samples had not been contaminated with soil. A significant increase in the content of crude ash relative to control was found in plants from all plots treated with slurry either on its own or together with soil conditioners or NPK fertilizers. This was confirmed by the research of Wróbel et al. [50], who also noted the impact of natural fertilizers on the content of crude ash in grassland forage.

According to Stachowicz [51], crude fiber content for ruminants should be at the level of 200–250 g·kg^{-1} DM, while Grzelak [52] recommends 180.0–220.0 g·kg^{-1} DM for dairy cows. However, other researchers on the nutritional value of grassland forage recommend much higher content, which in hay should range from 280.0 to 330.0 g·kg^{-1} DM [44,49,53]. In the present experiment, compared to plants treated with slurry only, the interaction of slurry with either soil conditioner also resulted in an increase in crude fiber content. However, a significant increase was only recorded on plots where slurry was applied together with Rosahumus. Truba et al. [54] observed a significant impact of soil conditioners on the concentration of crude fiber in *Dactylis glomerata* and *Lolium perenne* species. Wiśniewska and Stefaniak [21] confirmed the beneficial effect of slurry and bioproducts on the amount of crude fiber in *Festulolium braunii*.

According to nutritionists, the net energy concentration in forage should not be lower than 0.7 oat units in 1 kg^{-1} DM. In the present studies, it was at a fairly high level, although supplementing slurry with Humus Active or Rosahumus soil conditioner did not increase it compared to slurry used on its own. The concentration of net energy was similar to the values of this parameter in the studies conducted by Wiśniewska [55], who used manure and mineral-supplemented mushroom substrate.

Although slurry applied with soil conditioners did not contribute to an increase in the content of protein, crude ash or in net energy concentration in timothy, their amounts were comparable to those in grass treated with slurry combined with NPK fertilizers. Thus, slurry applied with soil conditioners did not adversely affect grass nutritional value.

The second important factor determining the yield and crop quality is weather conditions during the growing period. In the three-year experiment, the most favorable hydrothermal conditions were in the first (2015) year. In the second and third growing periods (2016 and 2017), months with low rainfall prevailed. Temperature and soil moisture significantly affected total protein and crude fiber content, as well as the net energy concentration.

The amount of meadow timothy biomass was not significantly different in the subsequent years of research although the first (2015) year was definitely more favorable for plant growth than the second and third. Probably the reason for a lack of differences in yields was that 2015 was the first year of grass growth and development, and the species does not grow rapidly in the first year after sowing. In the research of Olszewska [56] and Staniak and Kocoń [57], a decrease in soil moisture caused a decrease in meadow timothy yield, and the most favorable response to water deficiency was shown by its Karta variety. During the periods of water scarcity, meadow timothy accumulated larger amounts of crude fiber, while reducing both total protein content and net energy concentration. The opposite relationship was noted by Szkutnik et al. [46,47], while Olszewska [58] reported that a decrease in the amount of fiber caused by drought was dependent on the grass species.

The growth cycle had a significant impact on all forage parameters, except for crude ash. Recorded in the present research, the total protein and crude fiber content of meadow timothy in consecutive harvests was not confirmed by the research of Radkowski et al. [25], who recorded the most total protein in the third harvest and crude fiber in the first.

Soil analysis at the end of the experiment showed a significant increase in the content of nitrogen, phosphorus and potassium in response to treatment. Compared to the effect of slurry used on its own, and even together with NPK fertilizers, a significant increase in nitrogen content was noted in the soil treated with slurry together with the Humus Active soil conditioner. A high yield and high nitrogen content of plants treated with slurry and Humus Active may indicate a reduction in nitrogen losses caused by the interaction of both fertilizers.

The greatest accumulation of phosphorus was recorded in the soil fertilized with slurry and Rosahumus. Each soil conditioner used together with slurry had a similar effect on potassium content. It should be emphasized that although supplementing slurry with soil conditioners did not introduce such amounts of macronutrients into the soil as complementary mineral fertilizers, the content of nitrogen, potassium and phosphorus in the soil treated with soil conditioners was comparable to the effect of NPK fertilizers and sometimes greater.

5. Conclusions

Slurry applied together with Humus Active or Rosahumus significantly increased the yield of meadow timothy compared to the effects of slurry either applied on its own or together with mineral fertilizers. However, this did not translate into an increase in the total protein content of plants. The content of crude ash as well as the concentration of net energy in forage from plots fertilized with slurry supplemented with soil conditioners was not significantly higher than in plants treated with slurry on its own or with NPK fertilizers. As an effect of slurry interaction with Rosahumus, a significant increase in the content of crude fiber was noted compared to plants treated with slurry on its own or in combination with NPK fertilizers. Although the interaction of slurry and soil conditioners did not significantly increase the content of protein, crude ash and the concentration of net energy in plants compared to slurry applied on its own or supplemented with NPK, the values of these parameters did not adversely affect forage quality. Soil analysis after the experiment proved a significant effect of the interaction of slurry and soil conditioners on macronutrient accumulation. The interaction of slurry with Humus Active resulted in a significant increase in nitrogen accumulation in the soil compared to the interaction of slurry with NPK as well as to the effect of slurry on its own. The use of slurry with Rosahumus contributed to a significant increase in phosphorus accumulation in the soil in relation to slurry either applied on its own or supplemented with minerals. Compared to the effect of slurry applied on its own, a significant increase in the amount of potassium in the soil was noted after the application of slurry used with Rosahumus, Humus Active or NPK fertilizers. The present studies provide evidence that it is possible to supplement slurry with soil conditioners instead of mineral fertilizers to obtain a high timothy meadow yield of good quality. At the same time, a beneficial effect of Rosahumus and Humus Active on the accumulation of NPK in the soil was noted, which is probably due to the reduction of their losses. Thus, although the research results in some cases are not conclusive, they demonstrate that slurry applied to timothy does not need to be supplemented with mineral fertilizers because an addition of soil conditioners results in good quality forage with high yields and in an increase in the content of soil NPK nutrients.

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