

## EFFECTS OF SLURRY AND SOIL CONDITIONERS ON THE YIELD, PROTEIN AND ASH CONTENT IN ITALIAN RYEGRASS (*LOLIUM MULTIFLORUM* LAM)

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**Abstract.** The purpose of this research was to determine the effect of slurry applied on its own and supplemented with mineral fertilizers or soil conditioners (UGmax and Humus Active) on the yield of *Lolium multiflorum* and on its total protein and crude ash contents. The research was conducted on the basis of a two-year (2016-2017) field experiment in which *Lolium multiflorum* forage grass of the Dukat variety was used. For the two growing seasons the highest average yield of the grass was obtained from plots with slurry application and mineral fertilizers. However, interaction of slurry with soil conditioners resulted in a lower yield compared to the plot where slurry was used on its own. The highest total protein content was obtained to *Lolium multiflorum* treated with slurry supplemented with mineral fertilizers. The highest crude ash content (an average across growing seasons and treatments) was recorded in the biomass of the third harvest, with the lowest amount recorded in the first.

**Keywords:** *fertilization, feed value, forage grasses*

### Introduction

The primary nutrient that determines forage quality is protein. According to Brzóska and Śliwiński (2011) total protein is made up of true protein and non-protein nitrogen compounds. Kukułka and Kozłowski (2004) point out that the share of true protein in grass biomass is 80-95% of total protein, while non-protein nitrogen compounds constitute the other 5-20%. The content of this nutrient in forage is affected to the highest degree by the level of nitrogen fertiliser. However, the amount of nitrogen doses mainly increases the content of non-protein nitrogen in plants. For this reason, with intensive nitrogen application the share of true protein might be reduced by up to 50-60%.

According to Falkowski et al. (2000), total protein content in grass also depends on the species and even on the variety, habitat fertility with the level of nitrogen, the development stage of the plant, the weather, and the harvest.

In addition to total protein, crude ash content is equally important from the point of view of plant nutritional value (Kitczak et al., 2011). Forage mineral content is dependent on the species composition, the stage of plant development during harvest, and on weather conditions. Chemical elements required by animals in large quantities are potassium, phosphorus, magnesium, and calcium. Equally important but absorbed in small quantities are microelement such as copper, zinc, or manganese (Falkowski et al., 2000).

The research was aimed at assessing the yield of *Lolium multiflorum* biomass, as well as the content of total protein and crude ash as a result of slurry applied on its own and supplemented with soil conditioners or NPK mineral fertilisers.

Supplementing the slurry with biopreparations was supposed to show how it will affect the amount of biomass obtained as well as the content of protein and ash components in it. In addition, the experiment was to assess whether the addition of biological preparations to liquid manure is able to balance complementary mineral fertilization.

## Materials and methods

The research was carried out on the basis of a two-year (2016-2017) field experiment established at the experimental facility of the University of Natural and Sciences and Humanities in Siedlce, with three replications and random layouts. The area of an experimental plot was 4.5 m<sup>2</sup> (1.5 x 3.0 m).

The main experimental factor tested in the research was slurry used separately and supplemented with NPK mineral fertilizers or soil conditioners with the commercial names of UGmax and Humus Active. The following research units were set up: (1) control (no treatment); (2) slurry; (3) slurry + UGmax; (4) slurry + Humus Active; (5) slurry + NPK.

The effect of slurry, soil conditioners, and mineral fertilisers was tested on the forage grass of the *Lolium multiflorum* species of the Dukat variety. It was sown in autumn 2015 at the sowing standard of 18 kg ha<sup>-1</sup>. Slurry from dairy cows was used as natural fertiliser. It was applied each year in a total dose of 30 m<sup>3</sup> ha<sup>-1</sup> divided into three equal parts used before each growth cycle.

Slurry had dry matter concentration of 10%, with a narrow ratio of C:N (8:1), while the concentration of selected macronutrients was as follows (g kg<sup>-1</sup> DM): N-33.0, P<sub>2</sub>O<sub>5</sub>-16.0, K<sub>2</sub>O-16, MgO-10.0 and Ca-21.0. Soil conditioners, according to the Institute of Soil Science and Plant Cultivation in Puławy, Poland, improve soil properties. Their composition is shown in *Table 1*.

*Table 1. Composition of soil conditioners*

Soil conditioner	Macronutrients (g kg <sup>-1</sup> )						Micronutrients (mg kg <sup>-1</sup> )				Microorganism and others
	N	P	K	Ca	Mg	Na	Mn	Fe	Zn	Cu	
UGmax	1.2	0.2	2.9	-	0.1	0.2	0.3	-	-	-	Lactic acid bacteria, photosynthetic bacteria, Azotobacter, Pseudomonas, yeast, actinomycetes
Humus Active	0.2	1.3	4.6	3.0	0.5	-	15	500	3	1	Permanent active humus with beneficial microorganisms

Soil conditioners were used annually before the first growth cycle at doses recommended by the manufacturer, i.e. UGmax (Ecodarpol company) at 0.6 L ha<sup>-1</sup> and Humus Active (Bogdan company) at 50 L ha<sup>-1</sup>. The addition of biologicals slurry was supposed to increase the use of manure nutrients by reducing losses, especially nitrogen. In addition, both biopreparations alleviate the symptoms of drought. Mineral nitrogen-

phosphate-potassium fertilisers (NPK) were used in the following doses: N – 100, P (P<sub>2</sub>O<sub>5</sub>) – 80, K (K<sub>2</sub>O) – 120 kg ha<sup>-1</sup>. Mineral nitrogen fertilizers were used in the form of ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>), phosphorus in the form of triple granular superphosphate (Ca (H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>) and potassium in the form of potassium salt (KCl). Phosphorus was applied once a year before the first growth cycle, whereas nitrogen and potassium doses were divided into three equal parts: the first before the start of vegetation, the second and third before the second and third growth cycles.

The experiment was set up on the soil with granulometric composition of loamy sand, the order of anthropogenic soils, the type of culture earth soil, and the subtype of horticole (Polish Soil Classification, 2011). Chemical analysis of the soil found that carbon concentration in organic compounds (C<sub>org</sub>) was 14.50 g kg<sup>-1</sup> DM, with total nitrogen of 1.36 g kg<sup>-1</sup> DM. The ratio of C:N was 10.6:1, and the pH value was 6.7. The concentration of absorbable forms of phosphorus (170.00 mg kg<sup>-1</sup> DM) and magnesium (84.00 mg kg<sup>-1</sup> DM) was high, with moderate concentration of potassium (114.00 mg kg<sup>-1</sup> DM).

Hydrothermal conditions were determined on the basis of meteorological data from the Hydrological and Meteorological Station in Siedlce. In order to measure temporal variability of weather conditions and their effects on plant growth and development Sielianinov's hydrothermal coefficient (*Table 2*) was determined (Bac et al., 1993). It was calculated using the monthly sum of atmospheric precipitation (P) and the monthly sum of average daily air temperatures (Σt), applying the formula:  $K = P/0.1 \Sigma t$  (Skowera and Puła, 2004).

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

Year	Month						
	April	May	June	July	August	September	October
2015	1.36	1.87	1.64	0.59	1.92	0.64	0.12
2016	1.22	2.63	0.87	1.08	0.18	1.46	1.94
2017	2.88	1.15	1.08	0.45	0.96	1.92	1.90

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

The optimal value of this coefficient was the range of 1.3 < K ≤ 1.6 (Skowera and Puła, 2004). In the first year (2016) the optimum thermal and humidity conditions occurred only in the month of September, with severely wet May, while June, July, and August, the most important months for plant growth and development of plants, were dry, moderately dry, and extremely dry. In the second year of the experiment the period from May to August ranged from moderately dry to severely dry, and the optimum conditions were not recorded during any month of the growing season.

During the two-year experiment in each growing season three harvests of grass were collected. Immediately after each harvest fresh matter was weighed, and a sample of 0.5 kg was collected to determine the yield of dry matter and to perform chemical analyses. The yield of dry matter was determined with the drying method. Total protein and crude ash content was measured with the near-infrared spectroscopy method (LSDS), using the LSDFlex N-500 with the ready-to-use INGOT calibration for dry forage. The

method is described in detail in the Polish standard of PN-EN ISO 12099:2010 and in the literature (Burns et al., 2010; Reddersen et al., 2012).

Analysis of variance for a three-factor experiment was used to process the results statistically. The significance of the effect of various treatments on the value of the tested traits was checked with the Fisher-Snedecor F test. The statistical program of Statistica 6.0-2001 was used for calculations.

## Results

Dry matter yields (Table 3) of *Lolium multiflorum* showed a significant variation depending on the treatment applied and the harvest. In the first and second year the highest yield, 12,40 and 12.90 Mg ha<sup>-1</sup>, respectively, was obtained from the plot where slurry was supplemented with NPK fertilisers. The lowest biomass yield was on the control plot (8.70 in the first and 9.50 Mg ha<sup>-1</sup> in the second year.

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**Table 3.** Dry matter yield of *Lolium multiflorum* (Mg ha<sup>-1</sup>)

Growing season (B)	Harvest (C)	Treatments (A)					Means
		O	S	S + UGmax	S + HA	S + NPK	
2016	I	3.10	4.20	4.30	4.10	4.50	4.04
	II	2.90	3.50	3.50	3.80	4.40	3.62
	III	2.70	3.80	4.00	2.30	3.50	3.26
	Total	8.70	11.50	11.80	10.20	12.40	10.9
2017	I	3.60	4.50	4.30	4.40	4.70	4.30
	II	3.10	3.50	3.80	4.00	4.20	3.72
	III	2.80	4.30	2.40	2.60	4.00	3.22
	Total	9.50	12.30	10.50	11.00	12.90	11.24
<b>Means across growing seasons</b>		9.10	11.90	11.15	10.60	12.65	11.07
<b>Means across harvests</b>							
I		3.35	4.35	4.30	4.25	4.66	4.17
II		3.00	3.50	3.65	3.90	4.30	3.67
III		2.75	4.05	3.20	2.45	3.75	3.24
LSD <sub>0.05</sub> for:	A = 0.75	B = NS	C = 0.49				
	A/B = NS*	B/A = NS	A/C = NS				
	C/A = NS	B/C = NS	C/B = NS				

\*not significant

The amount of biomass in individual harvests, as an average for treatments, was the largest in the first one (4.04 and 4.30 Mg ha<sup>-1</sup>), but the smallest in the third (3.26 and 3.22 Mg ha<sup>-1</sup>). The biomass yield (average for years) was significantly higher only on the plot where slurry was supplemented with mineral NPK (12.65 Mg ha<sup>-1</sup>) than in plants where slurry was applied on its own (11.90 Mg ha<sup>-1</sup>).

The total protein content in grass dry matter (*Table 4*) was significantly differentiated across treatments and growing seasons. The largest average protein content was in plants treated with slurry supplemented with mineral NPK; in the first year it was 162.8 g kg<sup>-1</sup> DM, in the second 163.4 g kg<sup>-1</sup> DM. The smallest average protein content of *Lolium multiflorum* was recorded on control: 128.4 g kg<sup>-1</sup> in the first and 129.4 g kg<sup>-1</sup> in the second year of the experiment.

The addition of soil conditioners to slurry did not have a more favourable effect on protein content than the effect of slurry applied on its own. The mean protein content in plants treated with soil conditioners and slurry was lower than on plots treated with slurry only. Mean (across years and treatments) protein content decreased in consecutive harvests, and it was the largest in the first (155.4 g kg<sup>-1</sup>) and the smallest in the third (145.9 g kg<sup>-1</sup>).

**Table 4.** Protein concentration in *Lolium multiflorum* dry matter (g kg<sup>-1</sup>)

Growing season (B)	Harvest (C)	Treatments (A)					Means
		O	S	S + UGmax	S + HA	S + NPK	
2016	I	135.6	167.1	153.8	152.5	163.9	154.6
	II	123.8	156.3	162.5	158.7	169.5	154.2
	III	125.7	149.8	136.9	146.8	154.9	142.8
	Means	128.4	157.7	151.1	152.7	162.8	150.5
2017	I	132.5	168.2	160.1	168.7	168.1	159.5
	II	130.4	159.4	148.4	151.4	164.6	150.8
	III	125.4	158.6	159.2	154.9	157.6	151.1
	Means	129.4	162.1	155.9	158.3	163.4	153.6
<b>Means across growing seasons</b>		128.9	159.9	153.5	155.5	163.1	152.1
<b>Means across harvests</b>							
I		134.1	167.4	167.0	160.1	166.0	158.9
II		127.1	157.9	155.5	155.1	167.1	152.5
III		125.6	154.2	148.1	150.9	156.3	147.0
LSD <sub>0.05</sub> for:		A = 9.43	B = 6.19	C = NS			
		A/B = NS	B/A = NS	A/C = NS			
		C/A = NS	B/C = NS	C/B = NS			

Another ingredient in assessing feed quality is crude ash. Crude ash content in *Lolium multiflorum* dry matter (*Table 5*) significantly varied in both years. In the first year of research, the largest accumulation of crude ash (107.3 g kg<sup>-1</sup>) was recorded in the biomass of the grass treated with slurry only. In the second year, on average, the most crude ash was on plots with slurry and mineral fertilizers (119.5 g kg<sup>-1</sup>).

The smallest crude ash content in the first year of research was in plants treated with slurry and NPK (102.3 g kg<sup>-1</sup>), and in the second on the control plot (102.7 g kg<sup>-1</sup>).

Across harvests, in the first year the largest ash content was recorded in the biomass of the third one (113.3 g kg<sup>-1</sup>) and in the second one (114.4 g kg<sup>-1</sup>), with the lowest amount in the first harvest of the first year (97.3 g kg) and in third one in the second year (107.9 g kg). Crude ash content, the average of years and harvests, was the smallest on the control plot (103.6 g kg<sup>-1</sup>) and the largest in plants treated with slurry and mineral fertilizers (110.9 g kg<sup>-1</sup>).

**Table 5.** Crude ash concentration in *Lolium multiflorum* dry matter (g kg<sup>-1</sup>)

Growing season (B)	Harvest (C)	Treatments (A)					Means
		O	S	S + UGmax	S + HA	S + NPK	
2016	I	97.6	95.5	98.9	92.3	102.3	97.3
	II	103.7	102.6	99.7	105.6	99.9	102.3
	III	112.1	123.9	111.6	114.5	104.6	113.3
	Means	104.5	107.3	103.4	104.1	102.3	104.3
2017	I	89.5	110.1	119.8	115.6	129.4	112.9
	II	103.5	116.8	115.7	116.2	119.7	114.4
	III	115.0	110.8	100.6	103.8	109.3	107.9
	Means	102.7	112.6	112.0	111.9	119.5	111.7
<b>Means across growing seasons</b>		103.6	109.9	107.7	108.0	110.9	108.0
<b>Means across harvests</b>							
I		93.6	102.8	109.4	103.9	115.9	105.1
II		103.6	109.7	107.7	110.9	109.8	108.3
III		113.6	117.4	106.1	109.2	106.9	110.6
LSD <sub>0.05</sub> for:		A = NS	B = 7.02	C = NS			
		A/B = NS	B/A = NS	A/C = NS			
		C/A = NS	B/C = 9.94	C/B = 12.3			

## Discussion

Due to the fact that *Lolium multiflorum* is a short-term grass it can be used for alternating grassland. Studies have shown that its yields ranges from 9.10 to 12.65 Mg ha<sup>-1</sup>. According to Jankowski et al. (2008) *Lolium multiflorum* is a high-productivity species. This is also confirmed by the study of Borowiecki (2002a, b), according to who the *Lolium multiflorum* yield can range from 9 to 12 Mg ha<sup>-1</sup>.

In the present studies the best results in the form of increased biomass of the grass (an average across years and harvests) were recorded on plots where slurry was supplemented with mineral fertilizers. Olszewska (2008) points out that the yield is the basic criterion for assessing treatment effectiveness. A yield increase as a response to natural fertilizer applied to grassland was also recorded by Barszczewski et al. (2011). The present studies showed that slurry supplemented with soil conditioners increased the amount of biomass of the grass, and it was 16-20% greater than on the control plot, while slurry supplement with NPK fertilizers increased the yield by 40%.

According to Kryszak et al. (2012) as well as Jankowska-Huflejt (2012) periodic shortage of water reduces the yield of meadow plants below their potential.

In the first year (2016) the optimum thermal and humidity conditions were only in the month of September, with severely wet May, while June, July and August, which are the most significant months for the growth and development of plants, were dry, moderately dry, and extremely dry. In the second year of the experiment (2017), the period from May to August ranged from dry to very dry, and the optimum conditions were not recorded during any month of the growing season.

The optimum total protein content is very important from the point of view of grass nutritional value. According to Grygierzec (2012) the lowest protein content in forage, necessary for the proper digestion process, should range from 150 to 170 g kg<sup>-1</sup> DM. In contrast, according to Jankowska-Huflejt et al. (2011), *Lolium multiflorum* biomass should contain about 115 g kg<sup>-1</sup> DM. In the present experiment protein content was within the limits of 128.9 to 163.1 g kg<sup>-1</sup> DM, and it was undoubtedly the nitrogen introduced in the form of slurry and mineral fertilizer that increased it.

Kotlarz et al. (2010) as well as Kasperczyk (2004) confirm that the level of nitrogen fertilizer determines total protein content in forage. Many authors (Ciepiela, 2004; Jankowska et al., 2008; Szkutnik et al., 2012) point out that total protein content is not always increased in proportion to the applied nitrogen fertilizer. A lower total protein content in dry matter may be due to the high yield potential of the species and it can be caused by dilution of the content of this ingredient in the higher amount of biomass.

In the present experiment protein content in biomass depends on the harvest from which it originates. The dependence of the harvest impact on total protein content in grass was confirmed in the studies of Łyszczarz (2003) and Tonn et al. (2013). According to Juszczak and Rękorajski (2007) the components of crude ash are chemical elements whose adequate quantity in forage is necessary for the normal growth and development of animal organisms, while excessive content (above 150 g kg<sup>-1</sup> DM) may result from contamination of plant material with soil (Nazaruk et al., 2009).

Throughout the research crude ash content in *Lolium multiflorum* ranged from 89.5 g kg<sup>-1</sup> to 123.9 g kg<sup>-1</sup> and did not exceed the above value, which shows the purity of the plant material analysed. Kotlarz et al. (2010) and Grzelak and Bocian (2009) point out that low content of crude ash of 40 g kg<sup>-1</sup> DM in the forage may be related to low concentration of minerals in the soil and to species composition of the forage.

In the present experiment, the analysis of the content of this parameter clearly showed a significant effect of slurry application in comparison with the control plot. An increase in crude ash in meadow forage as a result of natural fertiliser application was also noted by Jankowska-Huflejt and Wróbel (2010); Barszczewski et al. (2010) as well as Wróbel et al. (2013).

In the present experiment in the first year crude ash content in *Lolium perenne* increased in subsequent harvests (from 97.3 to 113.3 g kg<sup>-1</sup>). In the second year a reverse trend was recorded. Barszczewski et al. (2010) found that ash content in meadow plants varied depending on harvest time.

The obtained test results clearly show that the supplementary use of biopreparations did not affect the increase of the grass grown or the protein content in it compared to the facilities where the slurry itself was used. Only the addition to the slurry of mineral fertilization improved the value of these parameters in relation to the operation of the slurry itself.

## Conclusions

1. The dry matter yield of *Lolium multiflorum* significant varied across treatments and harvests. In both years of the research the highest yield of the grass was on the plot treated with slurry and mineral fertilizers, and the interaction of slurry with soil conditioners resulted in a decrease in the yield compared to the slurry-only plot.
2. Total protein content in *Lolium multiflorum* forage was significantly dependent on the treatment and growing season. The largest total protein content was recorded in biomass from the plot where slurry was applied together with mineral fertilizers, but this was not significantly different from plants treated with slurry only.
3. The growing season had a significant impact on crude ash content in the grass. The greatest amount of ash (on average from growing seasons and treatments) was recorded in *Lolium multiflorum* of the third harvest, while the least crude ash in the first one.
4. The obtained research results showed a beneficial effect of the slurry interaction only with mineral fertilization, while the interaction with biopreparations did not bring the expected effects. The most likely cause could be adverse hydrothermal conditions in both growing seasons of the crop. Therefore, the assessment of the interaction of slurry with biopreparations requires further research.

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