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Magdalena Stancheva Petkova

Bioproductive and quality characteristics of perennial ryegrass (*Lolium perenne* L.) in a technological aspect

AUTHOR'S SUMMARY OF

dissertation for awarding the educational and scientific degree «*Doctor*»

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Scientific supervisor
Assoc. Prof. Dr. *Tatyana Ivanova Bozhanska*

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✓ The dissertation is written on 162 pages, contains 22 tables and 24 figures. The bibliography covers 207 literary sources, of which 66 are in Cyrillic and 141 are in Latin.

✓ The experimental work was conducted in the period 2020-2022 in the experimental field of the "Mountain meadows and forage production" Department at the Research Institute of Mountain Stockbreeding and Agriculture – Troyan.

✓ The numbering of the tables and figures in the dissertation abstract does not correspond to the numbering in the dissertation.

✓ The public defence of the dissertation will be held on 19. 10. 2023, in the RIMSA – Troyan at 11:00 AM, on a meeting of the Scientific Jury, appointed by Order No RD 05-130/19.07.2023.

✓ The materials of the present defence are available on the website of the Agricultural Academy – www.agriacad.bg and the Research Institute of Mountain Stockbreeding and Agriculture – Troyan – www.rimsa.eu

Scientific Supervisor
Assoc. Prof. *Tatyana Ivanova Bozhanska*

Reviewers:
Prof. *Boryana Churkova* PhD
Assos Prof. *Atanas Sevov* PhD

Scientific opinions:
Prof. *Anelia Katova* PhD
Prof. *Nurettin Tahsin* PhD
Assoc. Prof. *Tsenka Zhelyazkova* PhD

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1. *INTRODUCTION*

The development of animal husbandry in Bulgaria requires the formation of fodder mass with high quality. The application of modern practices (environmentally friendly, science-based and energy-efficient) related to the principles of sustainable agriculture in fodder production lead to a targeted impact on crop yields. The agro climatic challenges in the growing area have a significant impact on the optimal development and realization of the full plant potential. Obtaining fodder with high nutritional value and quality parameters, which satisfies the food needs of farm animals, depends on soil fertility and biological diversity in the grass stand. Therefore, the choice of fodder crops, adaptable and highly resistant (tolerant) to the environmental conditions is a prerequisite for high productivity, quality and ecological stability of plants.

2. *PURPOSE AND OBJECTIVES*

The research work aims to establish the possibilities for the production of ecologically clean forage biomass with high nutritional value in the conditions of the Central Balkan Mountain through independent and mixed cultivation of perennial ryegrass.

To achieve the goal, research was conducted to establish the suitability of Bulgarian and introduced cultivars of perennial ryegrass for the production of quality fodder; the impact of foliar feeding on the nutritional regime, productive potential and quality of perennial ryegrass forage mass; the opportunities of mixed cultivation of perennial ryegrass with legumes adapted for mountain areas and obtaining fodder with a high protein content for the needs of animal husbandry.

To achieve the goal, the following scientific tasks were completed:

1. Study on perennial ryegrass cultivars (*Lolium perenne* L.) in terms of productivity and fodder quality under the conditions of the Central Balkan Mountain.
2. Impact of foliar feeding with fertilizers on the yield, productive and qualitative indicators of perennial ryegrass (*Lolium perenne* L.).
3. Study on the productivity and economic qualities of perennial ryegrass (*Lolium perenne* L.) and legume forage grasses in monoculture and mixed grass stands.
4. Evaluation of the fodder quality obtained from sown grass stands, including energy nutritional value and *in vitro* digestibility of dry matter.

3. *MATERIAL AND METHODS*

In the period 2020-2022, three field experiments were conducted in the experimental field of Department of "Mountain meadows and forage production" at the Research Institute of Mountain Stockbreeding and Agriculture, Troyan. They are the following:

Experiment 1. Study on perennial ryegrass cultivars (*Lolium perenne* L.) in terms of productivity and fodder quality under the conditions of the Central Balkan Mountain.

The purpose of the experiment is to study the productivity and quality of fodder from Bulgarian and introduced cultivars of perennial ryegrass, grown on non-irrigated areas under mountain conditions. The experimental variants included eight cultivars of perennial ryegrass, such as:

1. Harmoniya (control – Bulgaria – BG – IFC-Pleven)
2. Bokser (Germany – DE; Poland – PL);
3. Belida (Denmark – DK);
4. Esquire (Germany – DE; Denmark – DK; Italy – IT; USA – US)
5. Nira (Poland – PL)
6. Nui (Great Britain – GB);
7. Tetrani (Bulgaria – BG – IFC-Pleven).
8. Tetramis (Bulgaria – BG – IFC-Pleven).

Experiment 2. Impact of foliar feeding with fertilizers on the yield, productive and qualitative indicators of perennial ryegrass (*Lolium perenne* L.).

The purpose of the experiment is to establish the impact of fertilizing with Nano Sulfur and Panamin AGRO, applied in different doses, on the productivity and quality of perennial ryegrass grown on non-irrigated areas under mountain conditions. The experimental variants are:

1. Control (Untreated);
2. Treatment with Nano sulfur (100 ml/da);
3. Treatment with Nano sulfur (200 ml/da);
4. Treatment with Panamin Agro (150 g/da);
5. Treatment with Panamin Agro (250 g/da).

Experiment 3. Study on the productivity and economic qualities of perennial ryegrass (*Lolium perenne* L.) and legume forage grasses in monoculture and mixed grasslands.

The purpose of the experiment is to determine the productivity and economic qualities of perennial ryegrass (*Lolium perenne* L.) grown in monoculture and mixed grass stands with meadow legume crops. The experimental variants included:

Monoculture grass stands:

1. Perennial ryegrass (*Lolium perenne* L.);
2. Red clover (*Trifolium pratense* L.);
3. White clover (*Trifolium repens* L.);
4. Bird's-foot-trefoil (*Lotus corniculatus* L.);

Mixed grass stands:

5. Perennial ryegrass (*Lolium perenne* L.) + Red clover (*Trifolium pratense* L.);
6. Perennial ryegrass (*Lolium perenne* L.) + White clover (*Trifolium repens* L.);
7. Perennial ryegrass (*Lolium perenne* L.) + Bird's-foot-trefoil (*Lotus corniculatus* L.).

Studied indicators:

- Yield of fresh and dry matter (kg/da);
- Botanical analysis of the grass stand (%);
- Grass stand height (cm);
- Chemical analysis:

✓ Crude fiber (CFr, g kg^{-1}) according to *Weende*; Crude protein (CP, g kg^{-1}) according to *Kjeldahl*; Crude fat (CF, g kg^{-1}) *Soxhlet*; Ash (g kg^{-1}); Moisture content (g kg^{-1}); Dry matter (DM, g kg^{-1}); Calcium (Ca, g kg^{-1}); Phosphorus (P, g kg^{-1}); NFE (nitrogen-free extractive substances).

- Biochemical analysis:

✓ Neutral detergent fibers (NDF, g kg^{-1}); Acid detergent fibers (ADF, g kg^{-1}); Acid detergent lignin (ADL, g kg^{-1}) – according to Van Soest and Robertson (1979); Degree of lignification (coefficient) – percentage ratio of ADL and NDF; Gross energy (GE, MJ/kg DM; Exchangeable energy (EE, MJ/kg DM); Feed units for milk (FUM in kg DM); Feed units for growth (FUG in kg DM) according to Todorov et al. (2010); *In vitro* dry matter enzyme digestibility (DMD, g kg^{-1}) according to Aufrere (1982).

- The following statistical analyzes were used:

✓ Variation analysis; correlation analysis; regression analysis; One-way analysis of variance (ANOVA), multiple comparison of means by least statistically significant difference ($LSD_{0.05}$).

✓ They were conducted using the following software products: Analysis Toolpak for Microsoft Excel 2010, Statgraphics Plus v.2.1 and STATSOFT Satistics for Windows 10.

4. *CLIMATE AND SOIL CHARACTERISTICS*

For the conditions of the region, the average annual temperature is 11.3°C, and the average temperature in the vegetation is 15.0°C (Fig. 1). Compared to the previous period (1990-2019), temperatures are higher. The average annual amount of precipitation is 610.3 mm, and the average vegetation amount is 445.2 mm. Compared to the previous period, the precipitation has lower quantitative values.

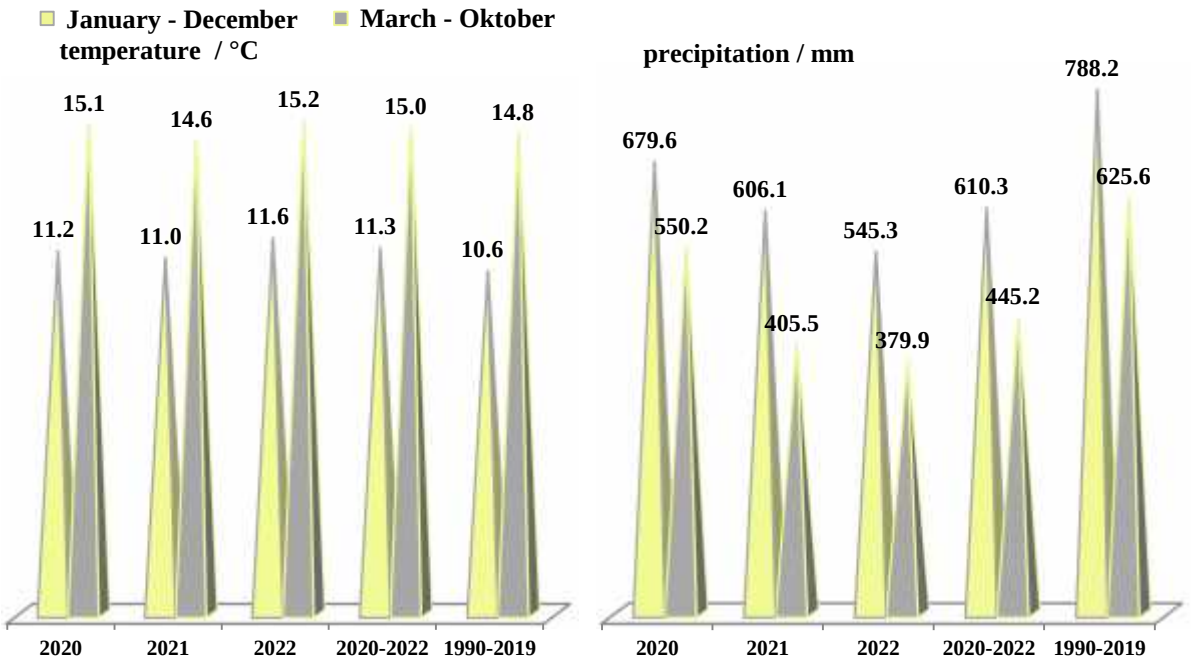


Figure 1. Climate characteristic of the area

The moisture supply for the three years of the experiment is not the same. In the first year, the precipitation amount is the highest (679.6 mm), and in the third the lowest (545.3 mm).

Soil conditions are important for the growth, development and yields of fodder crops. The soils in the experimental area are light gray, pseudo-podzolic. The humus content of the soil is low, with values of the indicator ranging from 1.25 to 1.40% in the 0-20 cm zone and from 1.24 to 1.39% in the 0-40 cm zone. Regarding the content of digestible phosphorus and potassium, the values range from 1.14 to 5.29 mg/100 (0-20) and from 0.26 to 2.29 mg/100 (0-40) – for phosphorus and from 20.97 to 23.93 mg/100 (0-20) and from 19.00 to 20.32 mg/100 (0-40) – for potassium. The availability of available nitrogen in the soil is low. In the soil horizon from 0-20 cm, where the root system of the plants is mainly located, the amount of the macroelement varies from 12.67 mg/100 to 21.31 mg/100, and in the horizon from 0-40 cm – 10.94 mg/100 to 12.67 mg/100. The soil reaction is acidic at pH_{H_2O} = 4.00-4.10 and pH_{kcl} = 3.60-3.90.

5. *RESULTS AND DISCUSSION*

5.1. Productivity, botanical composition and quality of fodder of perennial ryegrass cultivars (*Lolium perenne* L.) under the conditions of the Central Balkan Mountain.

The results of the conducted research summarize the difference in the performance of the studied cultivars, their adaptability and development under mountain soil and climate conditions.

5.1.1. *Plant height of perennial ryegrass cultivars.*

In the first year, the cultivars with proven (P <0.05) higher grass stands compared to the standard (Harmoniya) are: Nira, Nui, Tetrani, Tetramis.

Table 1. Plant heights of *Lolium perenne* L. cultivars, regrowths, by year and average over the period (cm)

Cultivars	2020			2021 I regrowth			2021 II regrowth			Average 2021			2022			2020-2022		
	X, cm	SD, cm	CV, %	X, cm	SD, cm	CV, %	X, cm	SD, cm	CV, %	X, cm	SD, cm	CV, %	X, cm	SD, cm	CV, %	X, cm	SD, cm	CV, %
Harmoniya (standard)	43.40	7.31	16.83	79.85	9.40	11.77	69.68	5.56	7.98	74.76	7.48	9.87	67.13	8.22	12.24	61.76	7.67	12.98
Bokser	41.60	5.53	13.30	82.03	6.61	8.06	66.18	9.16	13.84	74.10	7.89	10.95	62.70	8.11	12.93	59.47	7.18	12.39
Belida	36.90	5.75	15.60	80.08	8.52	10.64	60.05	8.67	14.44	70.06	8.59	12.54	61.35	9.43	15.38	56.10	7.92	14.51
Esquire	37.93	3.52	9.28	76.83	6.42	8.36	66.73	16.03	24.02	71.78	11.22	16.19	57.20	13.36	23.36	55.64	9.37	16.28
Nira	48.60	5.64	11.61	81.45	8.66	10.64	66.15	10.34	15.63	73.80	9.50	13.13	64.45	7.71	11.97	62.28	7.62	12.24
Nui	54.68	8.01	14.65	89.28	7.51	8.42	68.13	9.83	14.43	78.70	8.67	11.42	70.40	11.01	15.65	67.93	9.23	13.91
Tetrani	47.68	6.24	13.08	82.88	5.06	6.11	67.50	4.97	7.36	75.19	5.01	6.73	66.10	9.77	14.78	62.99	7.01	11.53
Tetramis	53.98	6.93	12.83	88.43	7.08	8.01	72.08	6.81	9.44	80.25	6.94	8.73	73.60	6.82	9.27	69.28	6.90	10.28
Average	***45.60***	***6.12***	***13.40***	***82.60***	***7.41***	***9.00***	***67.06***	***8.92***	***13.39***	***74.83***	***8.16***	***11.20***	***65.37***	***9.30***	***14.45***	***61.90***	***7.86***	***13.01***
CV, %	***13.41***			***8.97***			***13.30***			***10.91***			***14.23***			***12.70***		
LSD 0.05	***2.75***			***3.31***			***4.18***			***3.75***			***4.19***			***3.65***		

In the first cut of the second experimental year, all tested cultivars had the highest grass stand compared to their values for the entire study period. The plant stems of Nui (89.28 cm) and Tetramis (88.43 cm) cultivars were the tallest. The excess in the values of the indicator compared to Harmoniya cultivar is 9.43 and 8.58 cm. The results of the second regrowth of the same year indicate that only the Bulgarian tetraploid cultivar Tetramis (72.08 cm) had a higher grass stand compared to the diploid standard cultivar (69.68 cm).

In the third year, the genotypic differences in the indicator were the most pronounced (CV = 14.23). The tetraploid variety Tetramis had the highest grass stand height – 73.60 cm. Only in this cultivar a significant positive difference was observed compared to the diploid standard (67.13 cm). Opposite results were reported for Bokser – 62.70 cm, Belida – 61.35 cm and Esquire – 57.20 cm, and they were proven (P <0.05) inferior to the control variant (Harmoniya) –Table 1.

5.1.2. *Yield of fresh and dry matter of perennial ryegrass cultivars.*

In the first and third year, one mowing of the grass stand was conducted, whereas in the second year, two regrowths were harvested.

In the year of sowing, cultivar Nui had the highest fresh mass yield (2162.50 kg/da), exceeding cultivar Harmoniya significantly by 20.98% (Table 2). For the second and third experimental years, the highest values of the indicator were respectively found in Tetramis (6200.00 kg/da) and Tetrani (3375.00 kg/da). The differences compared to Harmoniya cultivar (5675.00 kg/da for 2021 and 3200.00 kg/da for 2022) are unproven. Expressed as a percentage, the values of the excess are 9.25 and 5.47%.

On average for the years of research, according to the fresh matter yield indicator (Table 2), the tetraploid Bulgarian cultivars Tetrani and Tetramis had the highest productivity (3741.70 kg/da) The excess in the values of the indicator compared to the diploid standard is 5.28%.

Table 2. Yield of fresh and dry matter (kg/da) of *Lolium perenne* L. cultivars

Variants	2020		2021		2022		2020-2022	
	kg/da	%	kg/da	%	kg/da	%	kg/da	%
Fresh matter								
Harmoniya (standard)	1787.50	100.00	5675.00	100.00	3200.00	100.00	3554.20	100.00
Bokser	1725.00	96.50	5762.50	101.54	2500.00	78.13	3329.20	93.67
Belida	1637.50	91.61	5462.50	96.26	3012.50	94.14	3370.80	94.84
Esquire	1825.00	102.10	6025.00	106.17	3350.00	104.69	3733.30	105.04
Nira	1925.00	107.69	5100.00	89.87	2562.50	80.08	3195.80	89.92
Nui	2162.50^{+}	120.98	5600.00	98.68	3337.50	104.30	3700.00	104.10
Tetrani	1912.50	106.99	5937.50	104.63	3375.00	105.47	3741.70	105.28
Tetramis	2012.50^{+}	112.59	6200.00	109.25	3012.50	94.14	3741.70	105.28
$LSD_{0.05}$	***314.80***	***17.60***	***645.50***	***11.40***	***525.80***	***16.40***	***398.10***	***11.20***
$LDS_{0.01}$	***426.60***	***23.90***	***874.80***	***15.40***	***712.50***	***22.30***	***539.50***	***15.18***
$LSD_{0.001}$	***571.30***	***32.00***	***1171.50***	***20.60***	***954.10***	***29.80***	***722.40***	***20.33***
η(SSg/SST)	***41%***		***42%***		***52%***		***43%***	
Dry matter								
Harmoniya (standard)	584.51	100.00	1725.67	100.00	1076.16	100.00	1128.40	100.00
Bokser	465.58	79.65	1720.57	99.70	799.00	74.25	995.00	88.18
Belida	487.98	83.48	1671.68	96.87	1013.41	94.17	1057.40	93.71
Esquire	559.00	95.63	1734.44	100.51	1075.69	99.96	1123.20	99.54
Nira	569.61	97.45	1472.18	85.31	777.98	72.29	939.80	83.28
Nui	688.32	117.76	1714.24	99.34	1081.68	100.51	1161.30	102.91
Tetrani	595.94	101.95	1619.13	93.83	1019.93	94.77	1078.40	95.57
Tetramis	575.78	98.51	1954.65	113.27	910.38	84.59	1146.40	101.59
$LSD_{0.05}$	***97.82***	***16.74***	***194.80***	***11.29***	***167.30***	***15.55***	***124.55***	***11.03***
$LDS_{0.01}$	***132.57***	***22.68***	***263.90***	***15.29***	***226.70***	***21.06***	***168.78***	***14.95***
$LSD_{0.001}$	***177.52***	***30.37***	***353.40***	***20.48***	***303.50***	***28.20***	***226.02***	***20.02***
η(SSg/SST)	***55%***		***54%***		***58%***		***49%***	

Dry matter yield in the year of sowing in Harmoniya cultivar was 584.51 kg/da (Table 2). Nui cultivar (688.32 kg/da) significantly exceeded the standard by 17.76% ($P < 0.05$).

The values during the second vegetation were the highest for the entire experimental period. The dry matter yield of Harmoniya cultivar is 1725.67 kg/da. Significantly ($P < 0.05$) higher yields were recorded by Tetramis cultivar (1954.65 kg/da).

In the third year, the tetraploid cultivar Tetramis (1146.40 kg/da) and the diploid cultivar Nui (1161.30 kg/da) had almost identical dry matter productivity compared to the standard (1128.40 kg/da), and Bokser and Nira cultivars were inferior to Harmoniya with significant differences of 11.82 and 16.72%.

Averaged over the experimental years, the dry matter yields of the tested cultivars ranged from 939.80 kg/da (Nira cv.) to 1161.30 kg/da (Nui cv.).

5.1.3. *Botanical composition of Lolium perenne L. cultivars.*

The results of the botanical analysis for the first year showed that the degree of weediness (Figure 2) in the tested cultivars ranged from 30.00% to 47.80%. In the conditions of the Central Balkan Mountain, Harmoniya cultivar has the highest relative share of the crop

(70.00%) in the mowed biomass, followed by Tetrani variety (69.80%).

In the second year (first regrowth), a low weed percentage was reported for all cultivars of perennial ryegrass. The results of the first mowing indicate that cultivars, such as Belida, Nui and Tetrani had the lowest proportion of weed vegetation (below 2.10%). In the second cut in 2021, the Bulgarian tetraploid cultivars Tetrani and Tetramis had the highest relative share of the crop, respectively 75.90% and 72.50%.

In 2022, Harmoniya was the most heavily weeded (25.00%), compared to the other tested cultivars, where the percentage of weed infestation was from 4.80% (Esquire cv.) to 19.60% (Tetrani cv.).

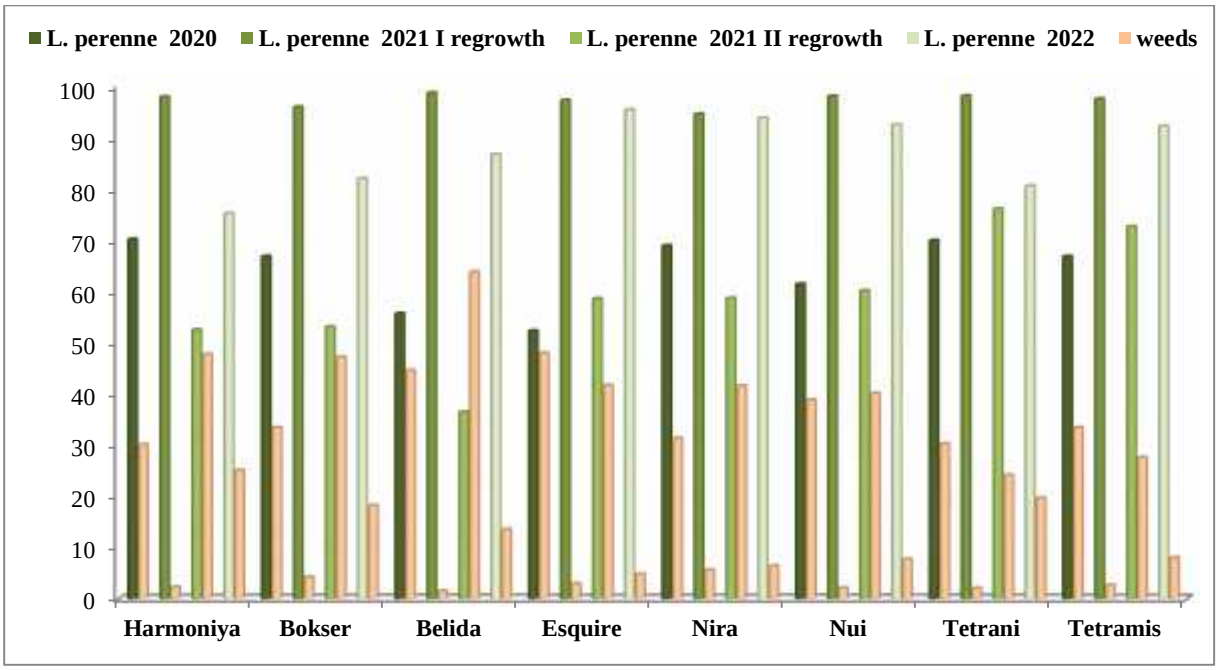


Figure 2. Botanical composition (%) of grass stands with *Lolium perenne* L. cultivars (2020-2022 г.)

The results of the botanical analysis, on average for the research period, allow us to summarize that the Bulgarian tetraploid cultivars Tetramis and Tetrani showed the highest resistance and adaptability under mountain conditions and register the highest relative share of the culture (over 79.00%) in the formed grass stands.

5.1.4. *Main chemical composition of Lolium perenne L. cultivars.*

The average value regarding the content of crude protein in the dry matter composition of the tested cultivars of perennial ryegrass was 131.79 g kg^{-1} DM (Table 3). Chemical analysis data for the 2020 and 2022 indicated that the crude protein content in the dry matter of Bokser had the highest values (135.78 and 138.20 g kg^{-1} DM, respectively). In 2021, as well as on average for the period, Belida cultivar recorded the highest crude protein content (149.50 and 144.27 g kg^{-1} DM).

The average value regarding the crude fiber content in the dry matter of perennial ryegrass cultivars is 374.56 g kg^{-1} DM, whereas the standard (Harmoniya cv.) is 355.30 g kg^{-1} DM.

Table 3. Main chemical composition (g kg^{-1} DM) of *Lolium perenne* L. cultivars by years and average for the period 2020-2022

Cultivars	DM	CP	CFr	CF	Ash	NFE	Ca	P
2020								
Harmonya (standard)	902.10	125.30	292.40	44.40	63.50	376.50	10.90	2.90
Bokser	899.80	135.78	296.40	38.90	67.30	361.42	12.14	2.27
Belida	905.00	133.82	288.50	32.30	78.60	371.78	12.22	3.57
Esquire	900.60	131.31	275.10	46.80	70.70	376.69	10.00	3.06
Nira	900.10	126.54	298.70	37.50	76.40	360.96	10.20	2.64
Nui	899.60	124.08	367.80	42.20	76.80	288.72	12.50	2.66
Tetrani	899.50	127.35	457.00	38.10	68.60	208.45	10.20	2.85
Tetramis	900.00	125.47	302.20	45.00	67.10	360.23	12.40	2.29
2021								
Harmony	930.85	125.95	468.00	18.15	48.70	270.05	10.15	2.95
Bokser	911.95	135.20	448.50	20.50	52.35	255.40	12.12	2.12
Belida	925.20	149.50	392.00	27.20	52.15	304.35	12.15	3.69
Esquire	928.95	144.50	374.90	28.70	56.90	323.95	10.90	2.18
Nira	918.55	141.65	400.90	19.15	51.05	305.85	10.05	2.85
Nui	920.85	125.10	378.15	24.40	53.55	339.55	12.55	2.83
Tetrani	916.40	127.95	370.10	23.10	56.20	338.95	11.10	2.95
Tetramis	915.85	130.85	401.00	34.45	52.35	297.15	12.10	2.89
2022								
Harmony	906.40	125.50	305.50	15.20	39.30	420.80	10.20	3.16
Bokser	909.10	138.20	393.50	16.20	39.30	322.00	18.90	2.01
Belida	910.00	122.70	361.50	11.00	39.10	375.70	21.10	3.78
Esquire	913.70	124.60	447.60	15.40	41.30	284.80	12.30	1.72
Nira	907.40	137.50	431.40	16.70	39.90	291.80	12.30	3.44
Nui	906.90	122.50	423.00	24.60	41.80	294.90	23.40	3.06
Tetrani	908.20	127.40	417.50	14.60	39.20	309.50	16.70	3.74
Tetramis	910.90	127.40	367.20	14.40	44.40	357.60	14.50	3.70
2020-2022								
Harmony	913.12	125.58	355.30	25.92	50.50	355.78	10.42	3.00
Bokser	906.95	136.39	379.47	25.20	52.98	312.94	14.39	2.13
Belida	918.47	144.27	357.50	28.90	60.97	326.83	12.17	3.65
Esquire	914.42	133.47	365.87	30.30	56.30	328.48	11.07	2.32
Nira	908.68	135.23	377.00	24.45	55.78	319.54	10.85	2.98
Nui	909.12	123.89	389.65	30.40	57.38	307.72	16.15	2.85
Tetrani	908.03	127.57	414.87	25.27	54.67	285.63	12.67	3.18
Tetramis	908.92	127.91	356.80	31.28	54.62	338.33	13.00	2.96
Average	***910.96***	***131.79***	***374.56***	***27.71***	***55.40***	***321.91***	***12.59***	***2.88***
MAX	***918.47***	***144.27***	***414.87***	***31.28***	***60.97***	***355.78***	***16.15***	***3.65***
MIN	***906.95***	***123.89***	***355.30***	***24.45***	***50.50***	***285.63***	***10.42***	***2.13***
Significance of the differences in the values of the indicator over the years	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	**P = 0.01**
Significance of differences in indicator values among cultivars	**P < 0.001**	**P < 0.001**	**P = 0.01**	P < 0.001	**P < 0.001**	P > 0.05	**P = 0.001**	P > 0.05

The biomass of Tetrani has the highest crude fiber content for the first experimental year (457.00 kg^{-1} DM), and for the second and third, respectively, Harmoniya (468.00 g kg^{-1} DM) and Esquire (447.60 g kg^{-1} DM).

With the highest average crude fat content are Tetramis (31.28 g kg^{-1} DM), Nui (30.40 g kg^{-1} DM), Esquire (30.30 g kg^{-1} DM) and Belida (28.90 g kg^{-1} DM). In the first year, the results of the indicator ranged from 32.30 g kg^{-1} DM (Belida) to 46.80 g kg^{-1} DM (Esquire). In the second year, the highest values were observed again in Esquire (28.70 g kg^{-1} DM), and in the third year for Nira (16.70 g kg^{-1} DM).

The content of mineral substances in the average dry weight of the tested cultivars varies from 50.50 g kg^{-1} DM (Harmoniya) to 60.97 g kg^{-1} DM (Belida) with an average value of 55.40 g kg^{-1} DM.

On average for the period, the highest amount of nitrogen free extractive substances was registered in the biomass of the standard – 355.78 g kg^{-1} DM with an average value – 321.91 g kg^{-1} DM.

The content of Ca varies from 10.42 g kg^{-1} DM (Harmoniya) to 16.15 g kg^{-1} DM (Nui), with an average value for the period – 12.59 g kg^{-1} DM. The results of the analysis indicate that the highest values of dry matter was found in Nui cv. for the entire study period (12.50 g kg^{-1} DM – 2020, 12.55 g kg^{-1} DM – 2021 and 23.40 g kg^{-1} DM – 2022 year).

The average phosphorus content in the dry matter of the studied cultivars for the period was 2.88 g kg^{-1} DM, whereas for the standard cultivar Harmoniya it was 3.00 g kg^{-1} DM. For the study period, Belida registered the highest values regarding the amount of the macroelement in the dry matter (3.57 g kg^{-1} DM for 2020; 3.69 g kg^{-1} DM for 2021 and 3.78 g kg^{-1} DM for 2022).

5.1.5. *Structural fiber components of the cell walls of forage mass of perennial ryegrass cultivars.*

For the study period, the average value of the indicator of neutral detergent fibers was 617.52 g kg^{-1} DM (Table 4). In the year of sowing, Nira (606.70 g kg^{-1} DM) had the highest value of NDF, whereas Tetramis (517.00 g kg^{-1} DM) had the lowest. In the second experimental year, an increase in the content of neutral detergent fibers was reported for all the tested variants, compared to the first year, with values ranging from 629.05 g kg^{-1} DM (Esquire cv.) to 714.65 g kg^{-1} DM (variety Bokser). In the third year, the highest and correspondingly lowest content of neutral detergent fibers was found in the biomass of Belida (688.80 g kg^{-1} DM) and Harmoniya (605.80 g kg^{-1} DM).

The content of acid detergent fibers in the dry matter of the tested cultivars of perennial ryegrass, on average for the experimental period, was 334.57 g kg^{-1} DM. In the year of sowing, in the third year and on average for the period, Belida had the lowest values of the indicator, respectively 317.00 g kg^{-1} DM, 190.20 g kg^{-1} DM and 275.38 g kg^{-1} DM . In the second year, the lowest value of the indicator was found in Bokser cv. (300.10 g kg^{-1} DM).

The average acid detergent lignin (ADL) content for the study period was 78.83 g kg^{-1} DM. The values of the indicator varied from 61.27 g kg^{-1} to 96.97 g kg^{-1} DM. For the years of research, as well as on average for the period, the lowest content of the hard-to-digest lignin fraction was found in Bokser cultivar with values of 70.50 g kg^{-1} DM (2020), 50.40 g kg^{-1} DM (2021), 62.90 g kg^{-1} DM (2022) and 61.27 g kg^{-1} DM (average for the period). In contrast, the grass mass of Esquire had the highest content of acid detergent lignin, the values of the indicator increasing with the age of the grass stand, namely: 90.20 g kg^{-1} DM (2020), 100.20 g kg^{-1} DM (2021) and 100.50 g kg^{-1} DM (2022).

Hemicellulose is a fully digestible polyoside, which significantly affects the biological sufficiency of the fodder. In the first year, Nira had the highest hemicellulose content (280.78 g kg^{-1} DM), and for all other cultivars, the values of the indicator exceeded the control by 4.55% (Tetramis) to 42.76% (Belida). In the second year, the highest content of hemicellulose was found in the dry matter of Bokser (414.55 g kg^{-1} DM), and for the last experimental year it was registered in Belida (498.60 g kg^{-1} DM).

Cellulose is a partially digestible fraction that affects the digestibility of dry matter and the nutritional value of the fodder. On average for the period, the polyoside content was 255.75 g kg^{-1} DM. Harmoniya cv. had the highest values in the first and second year, respectively 296.70 and 319.40 g kg^{-1} DM, and Tetrani in the third year (323.70 g kg^{-1} DM) and on average for the study period (323.70 g kg^{-1} DM).

Table 4. Fibrous structural components of cell walls and *in vitro* digestibility of dry matter (g kg^{-1} DM) of *Lolium perenne* L. cultivars, by year and average for the period 2020-2022

Cultivars	NDF	ADF	ADL	Hemicellulose	Cellulose	DMD according to Aufrere
2020						
Harmonya (standard)	565.30	382.90	86.20	182.40	296.70	613.70
Bokser	534.10	323.90	70.50	210.20	253.40	643.67
Belida	577.40	317.00	80.50	260.40	236.50	654.12
Esquire	554.20	329.40	90.20	224.80	239.20	643.48
Nira	606.70	325.92	80.60	280.78	245.32	643.24
Nui	560.20	348.30	81.80	211.90	266.50	621.07
Tetrani	550.80	329.40	73.03	221.40	256.37	643.76
Tetramis	517.00	326.30	60.50	190.70	265.80	643.31
2021						
Harmonya	674.30	390.20	70.80	284.10	319.40	598.32
Bokser	714.65	300.10	50.40	414.55	249.70	665.03
Belida	673.95	318.95	50.95	355.00	268.00	654.88
Esquire	629.05	362.00	100.20	267.05	261.80	610.19
Nira	672.55	312.60	80.15	359.95	232.45	618.03
Nui	645.60	359.70	90.30	285.90	269.40	610.06
Tetrani	636.95	370.65	90.75	266.30	279.90	610.84
Tetramis	640.65	379.70	80.95	260.95	298.75	603.18
2022						
Harmonya	605.80	304.70	80.20	301.10	224.50	665.14
Bokser	620.20	209.70	62.90	410.50	146.80	732.25
Belida	688.80	190.20	70.05	498.60	120.15	754.68
Esquire	629.20	282.00	100.50	347.20	181.50	677.20
Nira	627.30	272.50	100.10	354.80	172.40	687.20
Nui	645.90	422.00	80.77	223.90	341.23	576.10
Tetrani	626.00	385.90	80.30	240.10	305.60	598.90
Tetramis	623.90	485.70	79.15	138.20	406.55	511.60
2020-2022						
Harmonya	615.13	359.27	79.07	255.87	280.20	625.72
Bokser	622.98	277.90	61.27	345.08	216.63	680.32
Belida	646.72	275.38	67.17	371.33	208.22	687.89
Esquire	604.15	324.47	96.97	279.68	227.50	643.62
Nira	635.52	303.67	86.95	331.84	216.72	649.49
Nui	617.23	376.67	84.29	240.57	292.38	602.41
Tetrani	604.58	361.98	81.36	242.60	280.62	617.83
Tetramis	593.85	397.23	73.53	196.62	323.70	586.03
Average	***617.52***	***334.57***	***78.83***	***282.95***	***255.75***	***636.66***
MAX	***646.72***	***397.23***	***96.97***	***371.33***	***323.70***	***687.89***
MIN	***593.85***	***275.38***	***61.27***	***196.62***	***208.22***	***586.03***
Significance of the differences in the values of the indicator over the years	P > 0.05	P > 0.05	**P = 0.01**	**P < 0.05**	P > 0.05	P > 0.05
Significance of differences in indicator values among the cultivars	**P < 0.001**	P > 0.05	P > 0.05	**P < 0.01**	P > 0.05	P > 0.05

The biomass of cultivar Belida had the highest *in vitro* digestibility of dry matter in the first (654.12 g kg^{-1} DM) and the third (754.68 g kg^{-1} DM) year, as well as on average for the period (687.89 g kg^{-1} DM). In the second year, the lowest value of the indicator was found in Bokser (665.03 g kg^{-1} DM).

The average value for digestibility of the investigated perennial ryegrass cultivars is 636.66 g kg^{-1} DM. Belida (687.89 g kg^{-1} DM), Bokser (680.32 g kg^{-1} DM), Nira (649.49 g kg^{-1} DM) and Esquire (643.42 g kg^{-1} DM) were characterized with higher *in vitro* dry matter digestibility compared to the average for the experiment.

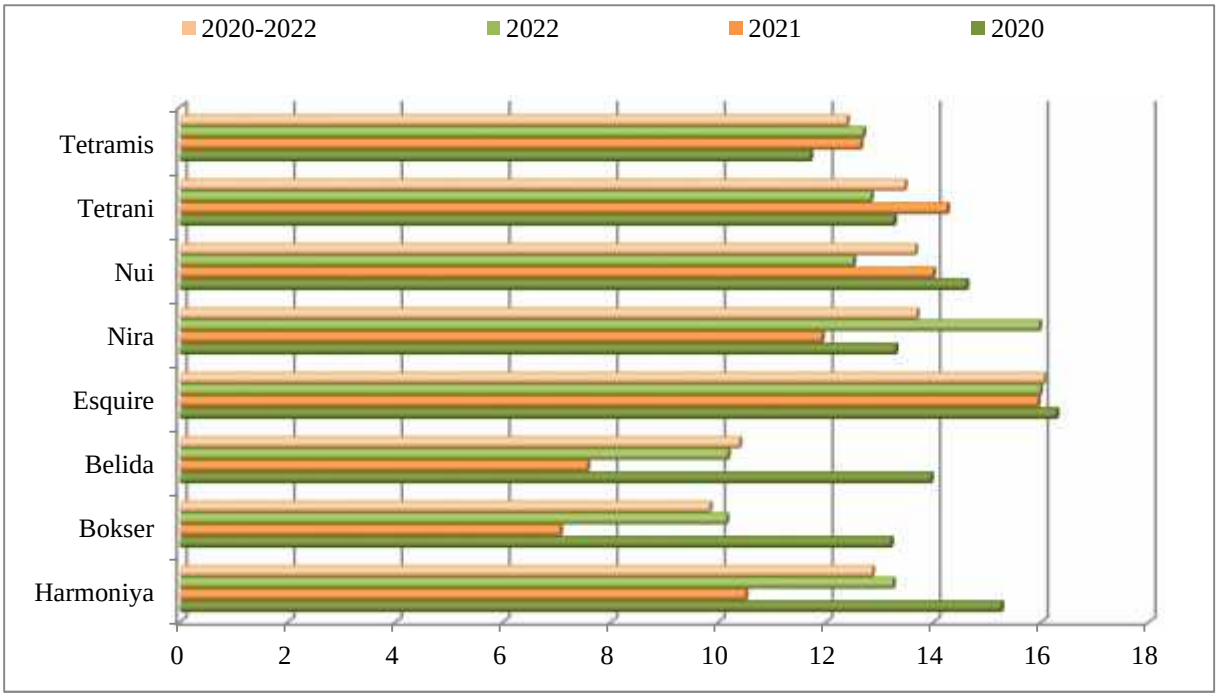


Figure 3. Degree of lignification of fodder of *Lolium perenne* L. cultivars, by years and average for the period (coefficient)

The degree of lignification in the studied cultivars of perennial ryegrass, expressed by the lignification coefficient, is 12.79 (Figure 3). Bokser (coefficient = 9.83) has a lower value compared to the control variant (coefficient = 12.85). In the year of sowing, the grass stand with Tetramis cultivar (coefficient = 11.70) had the smallest lignification coefficient. In the second (coefficient = 7.05) and third (coefficient = 10.14) years, Bokser had the lowest degree of lignification. Over the years of the experimental period (coefficient = 16.28 in 2020, coef = 15.93 in 2021 and coef = 15.97 in 2021) and average for the period (coefficient = 16.05 for the period 2020-2022) the biomass of cultivar Esquire had the highest degree of lignification.

5.1.6. *Potential energy nutritive value of fodder from perennial ryegrass cultivars.*

The average value for the experimental period according to the indicator of total energy value of the fodder for perennial ryegrass cultivars was 17.50 MJ/kg DM (Table 5). In the year of sowing, Belida had the lowest content of gross energy – 16.77 MJ/kg DM, whereas Tetrani – 17.57 MJ/kg DM had the highest. The results for the amount of gross energy in the second year had higher values compared to the first and third year in five cultivars (Bokser, Belida, Esquire, Tetramis and Harmoniya) of the total eight cultivars studied, with values of the indicator ranging from 17.74 (Bokser) up to 18.08 MJ/kg DM for Harmoniya cultivar. In the second year, Tetrani registered the lowest value of gross energy (17.44 MJ/kg DM), and in the third – Nira (17.92 MJ/kg DM).

The average value regarding the exchangeable energy (EE) in perennial ryegrass cultivars was 7.33 MJ/kg DM. Esquire (7.41 MJ/kg DM), Tetramis (7.42 MJ/kg DM), Belida (7.45 MJ/kg DM) and Harmoniya (7.50 MJ) had a higher amount of exchangeable energy compared to the average /kg DM). The excess in the values of the indicator is from 1.09% to

2.32%. In the first (7.64 MJ/kg DM) and the second (7.52 MJ/kg DM) biomass of the Esquire cultivar had the highest amount of exchangeable energy, and in the third year Harmoniya with 7.77 MJ/kg DM.

Table 5. Energy nutritional value of fodder from *Lolium perenne* L. cultivars, by year and average for the period 2020-2022

Cultivars	GE	EE	FUM	FUG
2020				
Harmoniya (standard)	17.17	7.63	0.70	0.64
Bokser	17.05	7.54	0.70	0.63
Belida	16.77	7.49	0.69	0.63
Esquire	17.04	7.64	0.71	0.65
Nira	16.81	7.42	0.68	0.62
Nui	17.14	7.06	0.64	0.57
Tetrani	17.57	6.66	0.60	0.52
Tetramis	17.12	7.52	0.69	0.63
2021				
Harmoniya	18.08	7.08	0.64	0.56
Bokser	17.74	6.96	0.63	0.55
Belida	17.98	7.44	0.68	0.60
Esquire	17.89	7.52	0.69	0.62
Nira	17.71	7.30	0.66	0.59
Nui	17.60	7.42	0.68	0.61
Tetrani	17.44	7.38	0.68	0.61
Tetramis	17.86	7.30	0.66	0.59
2022				
Harmoniya	17.13	7.77	0.72	0.66
Bokser	17.64	7.35	0.67	0.60
Belida	17.31	7.50	0.69	0.62
Esquire	17.78	7.08	0.64	0.56
Nira	17.92	7.23	0.66	0.58
Nui	17.72	7.16	0.65	0.58
Tetrani	17.60	7.20	0.65	0.58
Tetramis	17.36	7.44	0.68	0.62
2020-2022				
Harmoniya	17.46	7.50	0.69	0.62
Bokser	17.47	7.28	0.66	0.59
Belida	17.58	7.45	0.68	0.61
Esquire	17.57	7.41	0.68	0.61
Nira	17.48	7.32	0.67	0.60
Nui	17.49	7.21	0.66	0.59
Tetrani	17.54	7.08	0.64	0.57
Tetramis	17.45	7.42	0.68	0.61
Average	***17.50***	***7.33***	***0.67***	***0.60***
MAX	***17.58***	***7.50***	***0.69***	***0.62***
MIN	***17.45***	***7.08***	***0.64***	***0.57***
Significance of the differences in the values of the indicator over the years	P > 0.05	P > 0.05	P > 0.05	P > 0.05
Significance of differences in indicator values among cultivars	P < 0.001	P > 0.05	P > 0.05	P > 0.05

GE – gross energy MJ/kg DM; EE – exchangeable energy – MJ/kg DM; FUM – feed units for milk – units in kg of dry matter; FUG – feed units for growth – units in kg of dry matter.

Average values of feed units for milk (FUM) of the cultivars tested ranged from 0.64 in kg DM (Tetrani) to 0.68 in kg DM (Tetramis, Belida and Esquire). The grass stands of the standard (variety Harmoniya) recorded the highest number of FUM (0.69 in kg DM) in dry matter. In the first (0.71 in kg DM) and second (0.69 in kg DM) year, variety Esquire has the highest values of the indicator, and in the third variety – Harmoniya (0.72 in kg DM), as this is the highest value reported for the entire study period.

The feed units for growth (FUG) in the study years averaged 0.60 in kg DM. Harmoniya had the highest value of 0.62 in kg DM. Tetramis, Belida and Esquire also had a

higher FUG content (0.61 in kg DM). Esquire also had the highest FUG content in the first (0.65 in kg DM) and the second (0.62 in kg DM) year.

Harmoniya had the highest content of feed units for growth (0.66 in kg DM) and milk for the entire experimental period, making it suitable for feeding young animals.

5.2. Impact of foliar feeding with fertilizers on the yield, productive and qualitative indicators of perennial ryegrass (*Lolium perenne* L.).

5.2.1. *Plant height of perennial ryegrass after applied foliar feeding.*

Perennial ryegrass plants formed the highest grass stand in the variants with bio-fertilization with Panamin Agro (250 g/da) – Table 6. The harvested biomass had proven ($P < 0.05$) higher values of the indicator (by 4.05 cm) compared to the control (67.18 cm). For the study period, plant height in the variant ranged from 48.13 cm (2020) to 98.13 cm (2021).

Table 6. Plant heights of *Lolium perenne* L. with applied foliar feeding, by years (cm)

Variants	**2020**			**2021**			**2022**		
	X, cm	**SD, cm**	**CV, %**	**X, cm**	**SD, cm**	**CV, %**	**X, cm**	**SD, cm**	**CV, %**
Control	45.03	5.31	11.80	94.88	6.84	7.21	67.18	8.56	12.74
Nano Sulfur-100 ml/da	46.33	4.63	10.00	92.23	6.42	6.96	66.18	9.38	14.18
Nano Sulfur-200 ml/da	47.30^{+}	4.65	9.83	97.10	9.11	9.38	67.78	6.75	9.95
Panamin Agro-150 g/da	46.43	4.56	9.83	96.65	6.25	6.47	66.45	8.79	13.23
Panamin Agro-250 g/da	48.13^{+}	5.15	10.70	98.13^{+}	6.44	6.56	71.23^{+}	7.12	9.99
LSD 0.05	***2.15***			***3.13***			***3.61***		

In the year of sowing, the growth of perennial ryegrass (47.30 cm) treated with a higher dose of Nano Sulfur (200 ml) significantly exceeded ($P < 0.05$) the untreated control (45.03 cm). The data obtained with the application of the bioproduct Panamin Agro are also similar. Grass stands enriched with a higher dose of biopreparation (250 g/da) formed proven ($P < 0.05$) taller plant stems (48.13 cm) compared to the untreated control (45.03 cm). The coefficient of variation for the tested variants of the first experimental year is in the range from 9.83 to 11.80%.

For the second experimental year, only the height of the plants in the variant with the applied lower dose of the foliar fertilizer Nano Sulfur (100 ml) did not exceed the unfertilized control. The values of the indicator are lower by 2.65 cm. Treatment with a higher dose of mineral fertilizer greatly stimulates the growth of perennial ryegrass. In the variants treated with the bioproduct Panamin Agro, the trend is maintained. Variants with a higher dose (250 g/da) formed a proven ($P < 0.05$) higher grass stand with a coefficient of variation CV = 6.56%.

Only the foliar feeding variant with Panamin Agro in a dose of 250 g/da has a proven excess regarding the height of the plants. The values of the indicator exceeded the control by 6.03% at CV = 9.99%.

For the study period, biofertilization with Panamin Agro (250 g/da) had the most significant effect on the height characteristic of *Lolium perenne* L. plants.

5.2.2. *Yield of fresh and dry matter of perennial ryegrass after foliar feeding.*

On average for the period with the highest yield of fresh mass are the grass stands of *Lolium perenne* L. treated with Panamin Agro – 250 g/da (2020.80 kg/da) – Table 7.

The values of the indicator exceeded the control by 16.87% ($P < 0.05$). In the variants with Nano Sulfur, a slight advantage (with 112.50-166.60 kg/da) was observed in the amount

of fresh mass compared to the control (1729.20 kg/da), and in the grass stands treated with Panamin Agro (150 g/da), the values of the characteristic were lower compared to untreated ones.

Table 7. Yield of fresh and dry matter (kg/da) of *Lolium perenn*e L. after foliar feeding, by year and average for the period 2020-2022

Variants	2020		2021		2022		2020-2022	
	kg/da	%	kg/da	%	kg/da	%	kg/da	%
Fresh matter								
Control	1612.50	100.00	2350.00	100.00	1225.00	100.00	1729.20	100.00
Nano sulfur-100 ml/da	1800.00	111.63	2450.00	104.26	1275.00	104.08	1841.70	106.51
Nano Sulfur-200 ml/da	1512.50	93.80	2400.00	102.13	1775.00	144.90	1895.80	109.64
Panamin Agro-150 g/da	1550.00	96.12	2275.00	96.81	1312.50	107.14	1712.50	99.04
Panamin Agro-250 g/da	1700.00	105.43	2275.00	96.81	2087.50	170.41	2020.80^{+}	116.87
$LSD_{0.05}$	***466.55***	***28.93***	***334.78***	***14.25***	***332.33***	***27.13***	**268.93**	**15.48**
$LDS_{0.01}$	***654.88***	***40.61***	***469.92***	***20.00***	***466.48***	***38.08***	**377.49**	**21.73**
$LSD_{0.001}$	***924.54***	***57.34***	***663.42***	***28.23***	***658.56***	***53.76***	**532.93**	**30.67**
Dry matter								
Control	565.18	100.00	756.00	100.00	485.10	100.00	602.50	100.00
Nano Sulfur-100 ml/da	608.04	107.58	765.87	101.31	491.26	101.27	621.25	103.11
Nano Sulfur-200 ml/da	549.04	97.14	766.32	101.37	600.31	123.75	638.33	105.95
Panamin Agro-150 g/da	502.20	88.86	737.10	97.50	455.18	93.83	564.58	93.71
Panamin Agro-250 g/da	565.42	100.04	763.26	100.96	685.95	141.40	670.83	111.34
$LSD_{0.05}$	***155.84***	***27.52***	***109.70***	***14.51***	***112.86***	***23.27***	***88.47***	***14.68***
$LDS_{0.01}$	***218.75***	***38.63***	***153.98***	***20.36***	***158.42***	***32.66***	***124.18***	***20.61***
$LSD_{0.001}$	***308.82***	***54.54***	***217.38***	***28.74***	***223.65***	***46.11***	***175.31***	***29.10***

For the study period, no proven difference in the amount of dry matter was found between the variants with applied fertilizers and the untreated control of *Lolium perenne* L.

In the year of sowing, the highest yield of fresh (1800.00 kg/da) and dry matter (608.04 kg/da) was registered in the grass stands treated with Nano Sulfur (100 ml/da) followed by the variants with Panamin Agro-250 g/da (1700.00 – fresh matter and 565.42 – dry matter).

In the second year, the fertilizing again did not significantly affect perennial ryegrass productivity. The yield of fresh and dry matter in the variants with foliar feeding with Nano Sulfur – 100 and 200 ml/da was higher compared to the control by 50 to 100 kg/da, respectively – for the fresh matter and with 9.87 to 10.32 kg/da – for the dry matter. Grass stands of *Lolium perenne* L. with imported Panamin Agro had a lower productivity of grass matter compared to the unfertilized control.

In the third year, the effect of the applied foliar treatment affected to a higher extent the amount of perennial ryegrass biomass. The productivity of fresh and dry matter in the variants with Nano Sulfur (200 ml/da) and Panamin Agro (250 g/da) had significantly higher values. The excess in the values of the indicators is respectively by 44.90% and 23.75% (for the variants with Nano Sulfur – 200 ml/da) and by 70.41% and 41.40% (for the variants with Panamin Agro – 250 g/da).

5.2.3. *Botanical composition of monoculture crops with perennial ryegrass after foliar feeding.*

The botanical composition of the grass stand determines the quality and nutritional value of the fodder.

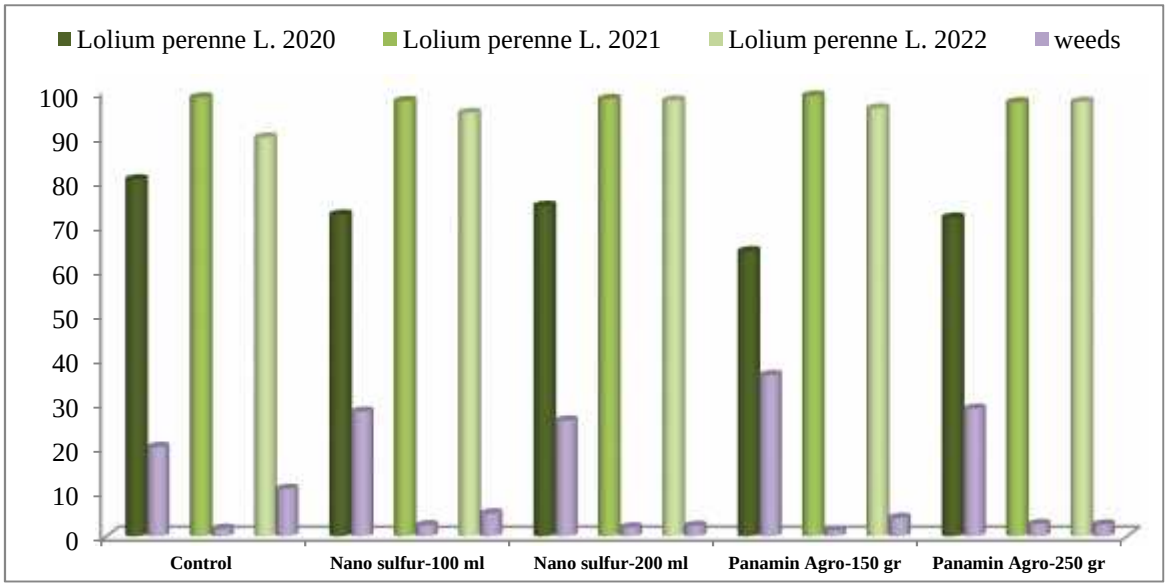


Figure 4. Botanical composition (%) of grass stands of *Lolium perenne* L. after foliar feeding (2020-2022)

In the year of sowing (Figure 4), the effect of the inorganic mineral fertilizer influenced to the highest extent the botanical composition of the grass stand. The percentage of *Lolium perenne* L. in the variants with Nano Sulfur varied from 72.09% (100 ml/da) to 74.07% (200 ml/da), and the excess compared to the control was 2.09 and 4.07%, respectively. In the variants with biofertilization, *Lolium perenne* L. had a share from 63.89% (150 g/da) to 71.43% (200 g/da) in the fodder mass. A minimum excess of 1.43% compared to the control was observed in the variants with a higher dose of Panamin Agro. The values regarding the degree of weediness in the crops with applied mineral and biofertilization vary from 25.93% (Nano Sulfur – 200 ml/da) to 36.11% (Panamin Agro – 150 g/da).

In the second year, perennial ryegrass had maximum share in the grassland. Panamin Agro compounds (150 g/da) ensured the highest (98.77%) share of *Lolium perenne* L. in the grass mass followed by the variants with Nano Sulfur – 200 ml/da (98.04%) and 100 ml/da (97.56%) and Panamin Agro – 200 g/da (97.30%).

In the year with the highest temperature and lowest precipitation amount (2022), the share of weed vegetation in the treated variants varied from 2.30% (Nano Sulfur – 200 ml/da) to 12.60% (untreated control). Perennial ryegrass responded to the effect of the applied fertilizers and registered the highest share in the grass stands with foliar feeding with Nano Sulfur – 200 ml/da (97.70%), followed by the variants with Panamin Agro – 250 g/da (97.40%); 150 g/da (95.90%) and Nano Sulfur – 100 ml/da (94.90%).

In the years of the experiment, perennial ryegrass occupied the highest share in the above-ground mass in the variants treated with higher doses of Nano Sulfur (200 ml/da) and Panamin Agro (250 g/da). The percentage share of the crop is respectively 89.94% and 88.71%, as the control was 79.95%. The specified variants had also the lowest degree of weed infestation. The mineral treatment with a rate of 100 ml/da ensured an 88.18% share of perennial ryegrass in the composition of the grass mass, and the bio-fertilization with Panamin Agro (150 g/da) – 86.18%.

5.2.4. *Main chemical composition of perennial ryegrass after foliar feeding.*

For the three experimental years, the highest crude protein content was found in the dry matter of the grass stands treated with Panamin-250 g/da (Table 8). The values of the indicator follow an increasing trend from the first (125.28 g kg^{-1}) to the third year (129.68 g kg^{-1}), and the excess compared to the control by years is respectively 28.62% (2020), with 35.92% (2021) and with 31.33% (2022).

Regarding the crude fiber content, only in the first experimental year, the grass stands of perennial ryegrass treated with the investigated preparations had lower values (from 10.30 to 34.21%) compared to the control (383.30 g kg^{-1}). In the second and third years, the crude fiber content of the fertilized variants varied from 391.20 g kg^{-1} (Panamin-250 g/da) to 439.50 g kg^{-1} (Nano Sulfur-100 ml/da), respectively, and from 281.30 g kg^{-1} (Panamin-150 g/da) to 393.50 (Nano Sulfur-200 ml/da). The excess compared to the control variants is from 0.18 to 12.55% (2021) and from 0.93 to 41.19% (2022). A predominance in the content of the fiber fraction was observed in the variants with foliar applied inorganic fertilizing, compared to the variants with organic fertilizing.

Table 8. Main chemical composition (g kg^{-1} DM) of *Lolium perenne* L. after foliar feeding by year and average for the period 2020-2022

Variants	DM	CP	CFr	CF	Ash	NFE	Ca	P
2020								
Control	892.30	97.40	383.30	37.50	80.30	293.80	14.80	3.09
Nano Sulfur-100 ml/da	891.90	124.99	285.60	36.50	85.80	359.01	18.10	5.32
Nano Sulfur-200 ml/da	890.50	115.43	311.30	30.80	85.20	347.77	16.60	2.74
Panamin-150 g/da	891.90	114.25	318.70	32.20	85.50	341.25	20.30	4.89
Panamin-250 g/da	888.70	125.28	347.50	36.40	86.70	292.82	30.40	1.90
2021								
Control	918.40	93.08	390.50	21.70	52.70	360.42	18.70	3.15
Nano Sulfur-100 ml/da	934.10	117.16	439.50	17.60	62.30	297.54	27.00	5.11
Nano Sulfur-200 ml/da	924.60	118.23	402.30	12.50	61.60	329.97	19.20	2.79
Panamin-150 g/da	930.50	121.46	396.00	17.40	60.70	334.94	31.90	4.91
Panamin-250 g/da	927.20	126.51	391.20	20.30	61.20	327.99	30.80	2.15
2022								
Control	913.80	98.74	278.70	17.60	44.50	474.26	16.60	3.24
Nano Sulfur-100 ml/da	930.60	123.15	362.30	16.70	62.70	365.75	26.10	4.12
Nano Sulfur-200 ml/da	918.40	125.08	393.50	19.40	67.30	313.12	18.90	2.86
Panamin-150 g/da	923.40	127.17	281.30	14.60	60.50	439.83	32.10	4.54
Panamin-250 g/da	919.90	129.68	291.20	18.40	61.80	418.82	31.20	2.88
2020-2022								
Control	908.17	96.41	350.83	25.60	59.17	376.16	16.70	3.16
Nano Sulfur-100 ml/da	918.87	121.77	362.47	23.60	70.27	340.77	23.73	4.85
Nano Sulfur-200 ml/da	911.17	119.58	369.03	20.90	71.37	330.29	18.23	2.80
Panamin-150 g/da	915.27	120.96	332.00	21.40	68.90	372.01	28.10	4.78
Panamin-250 g/da	911.93	127.16	343.30	25.03	69.90	346.54	30.80	2.31
Average	***913.08***	***117.17***	***351.53***	***23.31***	***67.92***	***353.15***	***23.51***	***3.58***
MAX	***918.87***	***127.16***	***369.03***	***25.60***	***71.37***	***376.16***	***30.80***	***4.85***
MIN	***908.17***	***96.41***	***332.00***	***20.90***	***59.17***	***330.29***	***16.70***	***2.31***
Significance of the differences in the values of the indicator over the years	***P = 0.05***	***P < 0.001***	*P > 0.05*	*P > 0.05*	***P = 0.01***	*P > 0.05*	***P < 0.001***	***P < 0.001***
Significance of differences in indicator values among variants	***P < 0.001***	*P > 0.05*	***P < 0.05***	***P < 0.001***	***P < 0.001***	***P = 0.05***	***P < 0.05***	*P > 0.05*

Data from the chemical analysis of crude fat content in the dry matter indicated that the average value for the experimental period was 23.31 g kg^{-1}. In the first experimental year, the values of the indicator for the treated variants ranged from 30.80 g kg^{-1} (Nano Sulfur-200

ml/da) to 36.50 g kg^{-1} (Nano Sulfur-100 ml/da). In the second year, again the lowest value of the indicator was registered in the variant with Nano Sulfur-200 ml/da (12.50 g kg^{-1}), and the highest in the grass stands with applied Panamin Agro-250 g/da (20.30 g kg^{-1}) In the third year, contrary to the previous two, with the highest crude fat content was the dry matter of the variant enriched with Nano Sulfur-200 ml/da (19.40 g kg^{-1}), and the lowest results were reported in the biomass foliarly treated with Panamin Agro-150 g/da (14.60 g kg^{-1}).

The content of mineral substances in the biomass of the treated variants of perennial ryegrass was higher compared to the controls.

Nitrogen free extractable substances are an empirically calculated indicator based on the amount of moisture, ash, crude protein, crude fat and crude fiber in the dry matter. In the first year, the variants with mineral fertilizing and those with bio-fertilization of Panamin Agro (150 g/da) registered higher values of the indicator with from 16.15% (Panamin Agro-150 g/da) to 22.20% (Nano Sulfur-100 ml/da) vs. control. In the second and third year, the results obtained are completely opposite. The investigated fertilizers affect the amount of the carbohydrate fraction in all treated variants, lowering the indicator values from 7.07% (Panamin Agro-150 g/da) to 21.13% (Nano Sulfur-100 ml/da) – for 2021 and from 7.83% (Panamin Agro-150 g/da) to 51.46% (Nano Sulfur-200 ml/da) – for 2022.

The analysis of the results indicates that the treatment of pasture ryegrass with Nano Sulfur (100 ml/da) increases the phosphorus content to the highest extent in the year of sowing and in the second year, when the values of the indicator exceed the unfertilized controls by 72.17% respectively and 62.22%.

On average for the study period, the grass stands of *Lolium perenne* L. with applied mineral and organic fertilizing registered a proven higher content of dry matter, crude protein, minerals and calcium. The values of the indicators in the treated variants vary accordingly:

- ✓ from 911.17 g kg^{-1} (Nano Sulfur – 200 ml/da) to 918.87 g kg^{-1} (Nano Sulfur – 100 ml/da) in the control 908.17 g kg^{-1} – for dry matter;
- ✓ from 119.58 g kg^{-1} (Nano Sulfur – 200 ml/da) to 127.16 g kg^{-1} (Panamin Agro – 250 g/da) in the control 96.41 g kg^{-1} – for the crude protein;
- ✓ from 68.90 g kg^{-1} (Panamin Agro – 150 g/da) to 71.37 g kg^{-1} (Nano Sulfur – 200 ml/da) in the control 59.17 g kg^{-1} – for mineral substances;
- ✓ from 18.23 g kg^{-1} (Nano Sulfur – 200 ml/da) to 30.80 g kg^{-1} (Panamin Agro – 250 g/da) in the control 16.70 g kg^{-1} – for the macroelement calcium.

The differences in calcium values were proven to be influenced by the age of the grass stand ($P < 0.001$) and the type of applied fertilizing ($P < 0.05$), and in the case of crude protein mainly by the factor of year and age of the grass crop ($P < 0.001$).

The differences in the amount of dry matter and mineral substances were highly influenced by the factors, such as year and age of the grass crop (respectively $P = 0.05$ and $P = 0.01$), as well as by the compositions and dose of the fertilizers ($P < 0.001$).

Grass stands with bio-fertilization are characterized by a lower crude fiber content than those with mineral fertilizing. The effect of Panamin Agro has been proven to reduce the concentration of the fiber fraction by up to 11.15% ($P < 0.05$).

On average for the study period, in the variants with applied inorganic and organic fertilizing, no excess was found in the content of crude fat, nitrogen free extractable substances and phosphorus, compared to the unfertilized control. Significance of differences in indicator values between variants, years and age of perennial ryegrass grass stand was demonstrated at $P < 0.05$ (for CF and NFE) and at $P < 0.001$ (for phosphorus).

5.2.5. *Structural fiber components of the cell walls of forage mass of perennial ryegrass after foliar feeding*

The results of the chemical analysis indicate that the average value of neutral detergent fibers is 641.79 g kg^{-1} in the control (603.94 g kg^{-1}) – Table 9. The variant treated with the biopreparation Panamin Agro (150 g/da) has the highest content of the indicator (668.82 g kg^{-1}) followed by the variant with applied inorganic fertilizer (Nano Sulfur – 200 ml/da) – 662.85 g kg^{-1}.

The content of acid detergent fiber in the dry matter of the untreated control was 373.93 g kg^{-1}. Two of the treated variants have higher values – (Nano Sulfur 200 ml/da – 396.73/ Panamin Agro 150 g/da – 394.03 g kg^{-1}), with an average value for the period – 374.25 g kg^{-1}.

The results of the chemical analysis indicated that for the years of study, the content of acid-detergent lignin (ADL) was the highest for the untreated control variant (111.64 g kg^{-1}), with an average value for the period (78.99 g kg^{-1}). The average values for the treated variants ranged from 64.55 g kg^{-1} (Panamin Agro 150 g/da) to 84.65 g kg^{-1} (Nano Sulfur-200 ml/da).

Table 9. Fibrous structural components of cell walls and *in vitro* digestibility of dry matter (g kg^{-1}) of *Lolium perenne* L. after foliar feeding, by year and average for the period 2020-2022.

Variants	NDF	ADF	ADL	Hemicellulose	Cellulose	DMD according to Aufrere
2020						
Control	560.40	341.50	109.00	218.90	232.50	632.25
Nano Sulfur-100 ml/da	550.11	333.70	61.93	216.41	271.77	632.77
Nano Sulfur-200 ml/da	580.42	391.80	81.25	188.62	310.55	598.66
Panamin-150 g/da	597.80	362.50	71.70	235.30	290.80	610.07
Panamin-250 g/da	620.65	340.60	63.70	280.05	276.90	632.41
2021						
Control	607.01	393.50	110.10	213.51	283.40	598.30
Nano Sulfur-100 ml/da	689.40	372.10	65.40	317.30	306.70	601.90
Nano Sulfur-200 ml/da	710.43	395.80	85.61	314.63	310.19	598.38
Panamin-150 g/da	713.60	413.90	68.20	299.70	345.70	576.37
Panamin-250 g/da	686.50	386.80	69.02	299.70	317.78	588.42
2022						
Control	644.40	386.80	115.81	257.60	270.99	588.32
Nano Sulfur-100 ml/da	619.60	301.60	71.22	318.00	230.38	655.24
Nano-sulfur-200 ml/da	697.71	402.60	87.10	295.11	315.50	587.12
Panamin-150 g/da	695.05	405.70	53.75	289.35	351.95	587.09
Panamin-250 g/da	653.70	384.80	71.10	268.90	313.70	598.26
2020-2022						
Control	603.94	373.93	111.64	230.00	262.30	606.29
Nano Sulfur-100 ml/da	619.70	335.80	66.18	283.90	269.62	629.97
Nano Sulfur-200 ml/da	662.85	396.73	84.65	266.12	312.08	594.72
Panamin-150 g/da	668.82	394.03	64.55	274.78	329.48	591.18
Panamin-250 g/da	653.62	370.73	67.94	282.88	302.79	606.36
Average	***641.79***	***374.25***	***78.99***	***267.54***	***295.25***	***605.70***
MAX	***668.82***	***396.73***	***111.64***	***283.90***	***329.48***	***629.97***
MIN	***603.94***	***335.80***	***64.55***	***230.00***	***262.30***	***591.18***
Significance of the differences in the values of the indicator over the years	P > 0.05	**P < 0.05**	**P < 0.001**	P > 0.05	**P < 0.05**	P > 0.05
Significance of differences in indicator values among variants	**P < 0.001**	**P < 0.05**	P > 0.05	**P < 0.05**	P > 0.05	P > 0.05

The hemicellulose content of the variants treated with the mineral preparation ranged from 266.12 (Nano Sulfur-200 ml/da) to 283.90 g kg^{-1} (Nano Sulfur-100 ml/da), with an average value of 267.54 g kg^{-1}. The values of the indicator in all treated variants exceeded the untreated control throughout the experimental period. The exception is the variants with Nano Sulfur in the first year, where lower results were reported.

The average cellulose content for the experimental period was 295.25 g kg^{-1} DM. The control variant had the lowest values for the entire study period (262.30 g kg^{-1}). For the experimental years, the cellulose content of the variants treated with a mineral preparation (Nano Sulfur) was higher at a dose of 200 ml/da, compared to the lower dose (100 ml/da) of the inorganic preparation. The results in the variants treated with the biopreparation Panamin Agro are the opposite, as the lower (150 g/da) administered dose exceeded the higher (250 g/da).

Forage digestibility analysis data indicated that values ranged from 591.18 (Panamin Agro-150 g/da) to 629.97 g kg^{-1} (Nano Sulfur-100 ml), with an average value of 605.70 g kg^{-1}. For all experimental years, perennial ryegrass treated with Nano Sulfur-100 ml/da had the highest values of the indicator, as follows: (2020 – 632.77; 2021 – 601.90; 2022 – 655.24 g kg^{-1}). The grass stands treated with a higher dose of Panamin Agro also had a higher dry matter digestibility (606.36 g kg^{-1}) on average for the experimental period compared to those treated with a lower dose (591.18 g kg^{-1}), in the control – 606.29 g kg^{-1}.

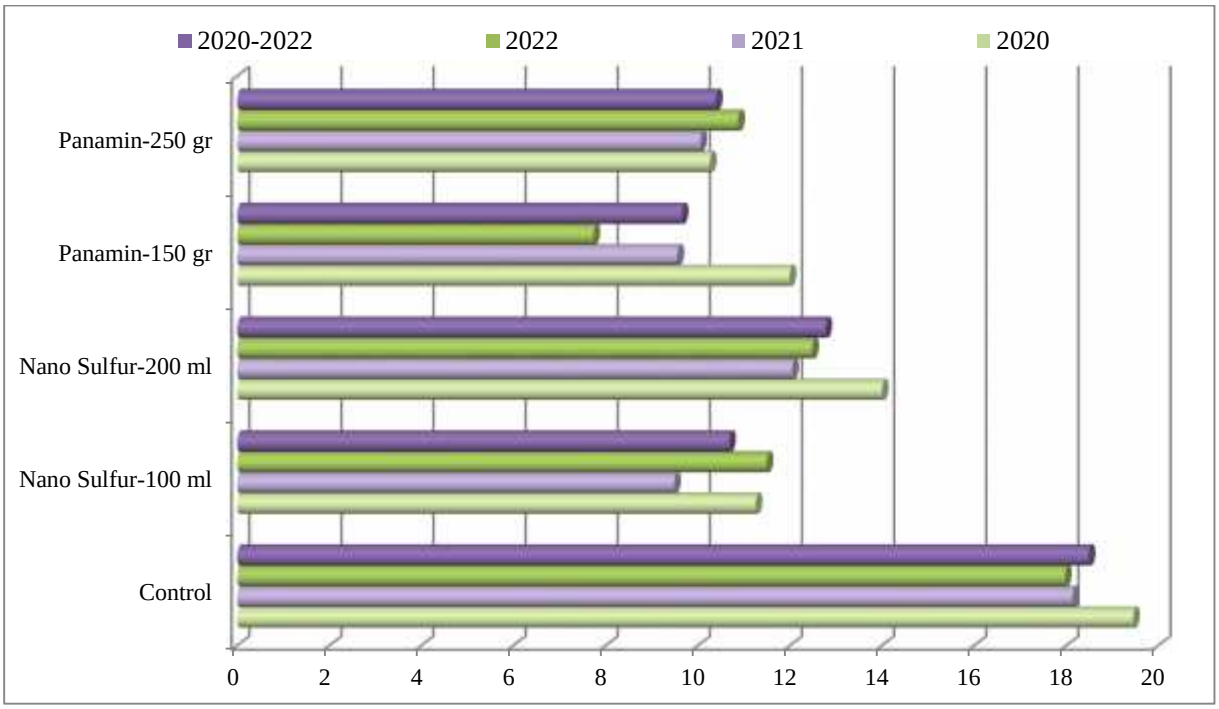


Figure 5. Lignification degree of perennial ryegrass forage after foliar feeding, by year and average for the period (coefficient)

According to the lignification degree indicator (Figure 5), the untreated control has the highest average value – (coefficient = 18.48). During the entire experimental period, perennial ryegrass treated with Nano Sulfur dose of 100 ml/da (10.68-mean value) had lower values compared to the higher dose of 200 ml/da (12.77-mean value). Lower average values for the degree of lignification was found in the variants with Panamin Agro at a dose of 150 g/da (coefficient = 9.65), compared to the higher dose of the biopreparation 250 g/da (coefficient = 10.39).

5.2.6. *Potential energy nutritive value of fodder from perennial ryegrass cultivars after foliar feeding.*

In the first experimental year, the total amount of energy (gross energy) released during the complete oxidation of nutrients was not positively affected by the applied mineral and organic fertilizing. The values of the indicator are by 0.84% lower (Panamin Agro-250 g/da) to 2.45% (Nano Sulfur-200 ml/da) compared to the unfertilized control (16.74 MJ/kg DM) (Table 10). The obtained results regarding the content of exchangeable energy and fodder units in the composition of the treated fodder matter for perennial ryegrass are completely opposite. The variants fertilized with Panamin Agro (250 g/da) had the lowest excess in the values respectively by 1.46% (for EE), 1.61% (for FUM) and 3.64% (for FUG), whereas the feed mass with added Nano Sulfur (100 ml/da) registered a maximum excess of 6.88% (for EE), 8.06% (for FUM) and 12.73% (for FUG) compared to the control.

In the second year, the empirically calculated gross energy was the highest (17.72 MJ/kg DM) in the grass stands with applied Nano Sulfur (100 ml/da). The excess in the values of the indicator is 2.19% compared to the control (17.34 MJ/kg DM). In Panamin Agro variants, the amount of gross energy also exceeded the control by 1.21-1.27%. Compared to the first experimental year, the amount of exchangeable energy and feed units in the composition of the dry matter with applied mineral fertilizing was lower compared to the unfertilized control. In the bio-fertilization variants, exchangeable energy content increased minimally from 0.27% Panamin Agro (250 g/da) to 0.41% Panamin Agro (150 g/da). The values of feed units for milk (0.67 in kg DM) and growth (0.60 in kg DM) were equal to those of the control variant.

In the third year, the data analysis shows a complete contrast to the first, regarding the values of the investigated indicators. All variants with applied mineral and organic fertilizing registered a higher amount of gross energy in dry matter from 0.30% Panamin Agro (150 g/da) to 2.72% Nano Sulfur (100 ml/da) compared to the control, and values for exchangeable energy and the number of feed units are lower. In the variants with foliar feeding by Nano Sulfur (200 ml/da), the values of energy (EE), which directly affects the life processes and productivity of the animals, as well as the feed units for milk and growth were lower compared to the control, respectively with up to 10.77% (for EE), 13.85% (for FUM) and 18.97% (for FUG).

The variants with foliar feeding by Nano Sulfur and Panamin Agro, had values of energy (EE), which directly affects the life processes and productivity of animals, as well as feed units for milk and growth, as their values were lower compared to the control (7.92 MJ/kg DM – EE, 0.74 kg of dry matter – FUM and 0.69 kg of dry matter – FUG). In the first case, at a norm of 200 ml/da, the decrease in the values of the indicators is up to 10.77% (for EE), 13.85% (for FUM) and 18.97% (for FUG), and in the second case with an imported norm of 250 g/da with up to 2.06% (for EE), 2.78% (for FUM) and 4.55% (for FUG) respectively.

Averaged over the period, the amount of gross energy in the foliar treatment variants ranged from 16.97 MJ/kg DM (Panamin Agro-150 g/da) to 17.18 MJ/kg DM (Nano Sulfur-100 ml/da). The maximum excess of the values compared to the control (17.00 MJ/kg DM) and the average of the indicator (17.04 MJ/kg DM) is by 0.82-1.06%. The difference in the values of the indicator between the variants with inorganic and organic fertilizing is significant ($P < 0.001$).

The difference in the number of feed units for milk and growth in the grass stands with imported Nano Sulfur and Panamin Agro was also proven to be highly dependent on the composition and rate of applied fertilizing ($P < 0.05$).

Table 10. Energy nutritional value of fodder from *Lolium perenne* L. after foliar feeding, by year and average for the period 2020-2022.

Variants	GE	EE	FUM	FUG
2020				
Control	16.74	6.83	0.62	0.55
Nano Sulfur-100 ml/da	16.43	7.30	0.67	0.62
Nano Sulfur-200 ml/da	16.34	7.13	0.66	0.60
Panamin -150 g/da	16.41	7.10	0.65	0.59
Panamin -250 g/da	16.60	6.93	0.63	0.57
2021				
Control	17.34	7.30	0.67	0.60
Nano Sulfur-100 ml/da	17.72	7.11	0.65	0.57
Nano Sulfur-200 ml/da	17.34	7.20	0.66	0.59
Panamin-150 g/da	17.55	7.33	0.67	0.60
Panamin-250 g/da	17.56	7.32	0.67	0.60
2022				
Control	16.92	7.92	0.74	0.69
Nano Sulfur-100 ml/da	17.38	7.48	0.69	0.62
Nano Sulfur-200 ml/da	17.28	7.15	0.65	0.58
Panamin-150 g/da	16.97	7.85	0.73	0.68
Panamin-250 g/da	17.02	7.76	0.72	0.66
2020-2022				
Control	17.00	7.35	0.68	0.61
Nano Sulfur-100 ml/da	17.18	7.30	0.67	0.60
Nano Sulfur-200 ml/da	16.99	7.16	0.65	0.59
Panamin-150 g/da	16.97	7.43	0.68	0.62
Panamin-250 g/da	17.06	7.34	0.67	0.61
Average	***17.04***	***7.31***	***0.67***	***0.61***
MAX	***17.18***	***7.43***	***0.68***	***0.62***
MIN	***16.97***	***7.16***	***0.65***	***0.59***
Significance of the differences in the values of the indicator over the years	P > 0.05	P > 0.05	P > 0.05	P > 0.05
Significance of differences in indicator values among variants	**P < 0.001**	**P = 0.01**	**P < 0.05**	**P < 0.05**

GE – gross energy MJ/kg DM; EE – exchangeable energy – MJ/kg DM; FUM – feed units for milk – units in kg of dry matter; FUG – feed units for growth – units in kg of dry matter.

The fodder mass of perennial ryegrass enriched with Panamin Agro (150 g/da) had the highest exchangeable energy content (7.43 MJ/kg DM). The excess in the values is by 1.09% (compared to the control) and by 1.64% (compared to the average value of the indicator).

5.3. Study of the productivity and economic qualities of perennial ryegrass (*Lolium perenne* L.) and forage legumes in monoculture and mixed grass stands.

5.3.1. *Height of perennial ryegrass and forage legumes in monoculture and mixed grass stands.*

For the study period, the average height of perennial ryegrass (*Lolium perenne* L.) in the monoculture grass stands ranged from 43.40 cm (2020) to 71.48 cm (2021) – Figure 6. The highest values of the indicator for the entire experimental period (81.05 cm) was registered in the first cut of the second harvest year.

The average height of the monoculture grass stands of legume meadow crops has optimal values for the foot-hill conditions of the Central Balkan Mountain, as for red clover the variation is from 34.13 cm (2020) to 67.05 cm (2021), and for white clover is from 22.80 cm (2022) to 36.99 cm (2021). In the second year, clovers had maximum values regarding plant height, both in the biomass of monoculture crops and in mixtures with perennial ryegrass (59.64 cm – red clover and 27.60 cm – white clover).

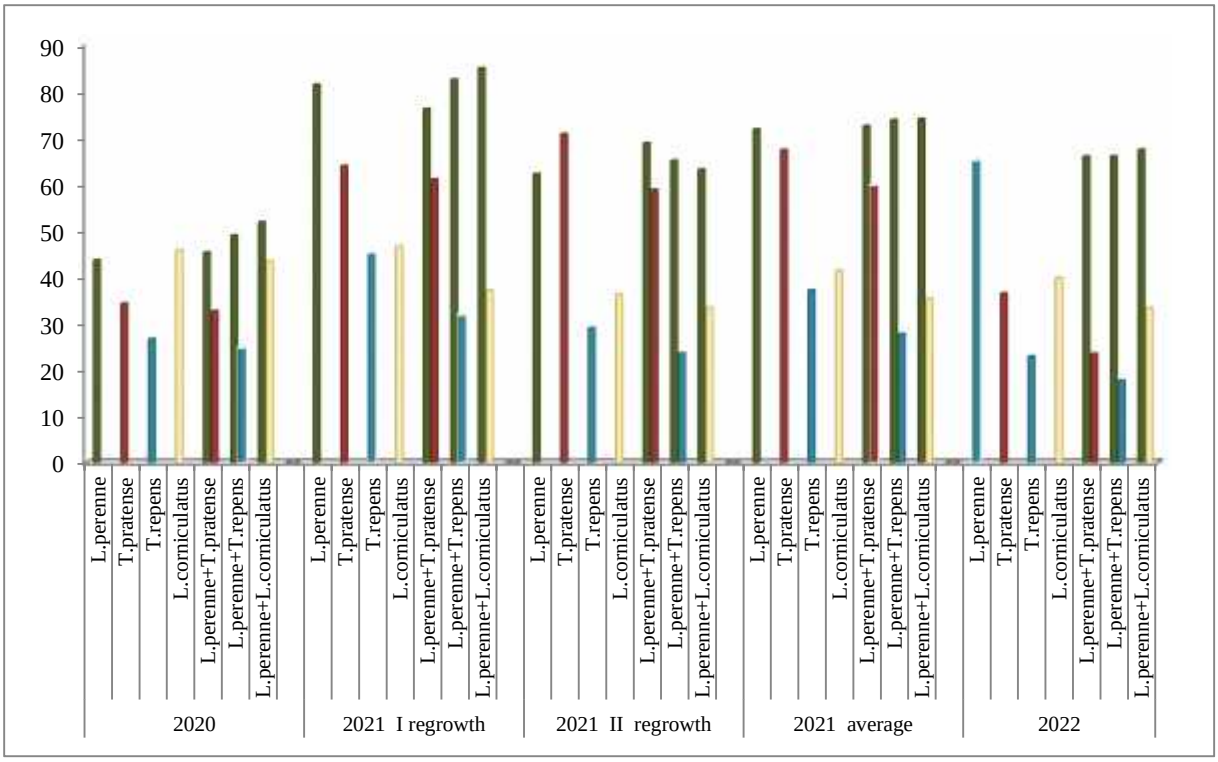


Figure 6. Height of perennial ryegrass and forage legumes in monoculture and mixed grass stands (cm), for the period 2020-2022

This trend is also preserved for the wheat component in the mixed grass stands. Perennial ryegrass also had maximum values in the second experimental year, when the plants were taller, respectively, from 6.52 cm (2022) to 27.15 cm (2020), in mixtures with red clover, from 7.78 cm (2022) to 27.15 cm (2020) in the mixtures with white clover and from 6.63 cm (2022) to 22.18 cm (2020) in the mixtures with bird's-foot-trefoil.

In the mixtures with white clover, the average height of perennial ryegrass plants was from 48.73 cm (2020) to 73.48 cm (2021), as in the first regrowth of the second experimental year, the grass crop registered a maximum value of 82.18 cm.

Perennial ryegrass is very competitive, which suppresses legume crops and they do not develop their full potential. For the entire study period, the perennial ryegrass in the mixed crops appeared as the dominant species according to the plant height indicator. In 2020, 2021 and 2022, the differences in the minimum and maximum value of the indicator are respectively from 0.70 cm to 1.65 cm in the two-component mixture *Lolium perenne* L. + *Trifolium pratense* L. and from 2.12 cm to 8.15 cm in the mixture *Lolium perenne* L. + *Lotus corniculatus* L.

5.3.2. *Yield of fresh and dry matter from monoculture and mixed grass stands of perennial ryegrass and forage legumes.*

Yield of fresh matter from monoculture and mixed grass stands of perennial ryegrass and forage legumes.

In the year of sowing, the yield of fresh matter in monoculture grass stands with perennial ryegrass (1837.50 kg/da) exceeded that of mixtures with red clover (1712.50 kg/da) and white clover (1725.00 kg/da), respectively with 7.30% and 6.52% (Table 11).

In the first year, the mixture of grass crop with *Lotus corniculatus* L. had the highest fresh matter productivity (1987.50 kg/da). The excess compared to mixtures with *Trifolium*

species is from 15.22% (in the mixture *Lolium perenne* L. + *Trifolium repens* L.) to 16.06% (in the mixture *Lolium perenne* L. + *Trifolium pratense* L.).

In the monoculture spring regrowths of the second year, perennial ryegrass registered a higher yield of fresh matter compared to the mixed ones. The excess in the values of the indicator are respectively 4.76% (in the mixture *Lolium perenne* L. + *Trifolium pratense* L.), 5.80% (in the mixture *Lolium perenne* L. + *Lotus corniculatus* L.) and 10.76% (in the mixture *Lolium perenne* L. + *Trifolium repens* L.). In contrast, the monoculture summer regrowths of the grass crop have lower values of the indicator from 21.57% (in the mixture *Lolium perenne* L. + *Lotus corniculatus* L.) to 43.14% (in the mixture *Lolium perenne* L. + *Trifolium pratense* L.) compared to those of the two-component mixtures.

In general, there was no significant difference in the fresh matter yield in the second year between monoculture grass stands of perennial ryegrass and mixtures, as well as between monoculture grass stands of legume crops. The highest productivity was found in red clover (6600.00 kg/da), followed by white clover (5750.00 kg/da) and bird's-foot-trefoil (5475.00 kg/da). This trend is also preserved in the mixed grass stands. The mixture of *Lolium perenne* L.+ *Trifolium pratense* L. (8525.00 kg/da) had the highest values, whereas *Lolium perenne* L. with *Trifolium repens* L. had the lowest (8025.00 kg/da).

Table 11. Yield of fresh matter from monoculture and mixed grass stands of perennial ryegrass and forage legumes, by years (kg/da)

Variants	2020	2021			2022
		I regrowth	II regrowth	Total	
Lolium perenne L.	1837.50	7975.00	637.50	8613.50	2225.00
Trifolium pratense L.	1500.00	5075.00	1525.00	6600.00	2187.50
Trifolium repens L.	1787.50	4350.00	1400.00	5750.00	2787.50
Lotus corniculatus L.	1825.00	4400.00	1075.00	5475.00	2562.50
Lolium perenne L.+ *Trifolium pratense* L.	1712.50	7612.50	912.50	8525.00	2837.50
Lolium perenne L. + *Trifolium repens* L.	1725.00	7200.00	825.00	8025.00	2912.50
Lolium perenne L. + *Lotus corniculatus* L.	1987.50	7537.50	775.00	8312.50	2512.50
$LSD_{0.05}$	*340.00*	*1646.23*	*340.00*	*2018.13*	*740.75*
$LSD_{0.01}$	*462.90*	*2241.30*	*590.00*	*2772.29*	*1008.52*
$LSD_{0.001}$	*624.40*	*3023.36*	*796.00*	*3792.83*	*1360.41*

Significant differences in the yield of fresh mass ($P < 0.05$) was observed among the variants of clover and bird's-foot-trefoil (grown as monocultures) with their mixtures with perennial ryegrass. The excess in the values of the indicator is by 29.17% (in the mixture *Lolium perenne L. + Trifolium pratense* L.); 39.57% (in the mixture *Lolium perenne* L. + *Trifolium repens* L.) and 51.83% (in the mixture *Lolium perenne* L. + *Lotus corniculatus* L.) in favour of mixed crops.

In the third year, the fresh mass in legume monocultures varied from 2187.50 kg/da (*Trifolium pratense* L.) to 2787.50 kg/da (*Trifolium repens* L.). The annual yield of fresh mass from the grass stands of perennial ryegrass is 2225.00 kg/da. Its association with white clover resulted in obtaining the highest yield of fresh mass (2912.50 kg/da) for 2022. The mixture of *Lolium perenne* L. + *Lotus corniculatus* L. (2512.50 kg/da) had the lowest values of the indicator. This is the only mixture whose productivity of fresh mass is lower compared to the yield of the monoculture grass stands of the legume component (2562.50 kg/da).

Averaged over the period (Figure 7) of the study, the yield of fresh mass in the monoculture grass stands with forage legumes (3429.17 kg/da – *Trifolium pratense* L., 3441.67 kg/da – *Trifolium repens* L. and 3287.50 kg/da – *Lotus corniculatus* L .) has proven ($P < 0.001$) lower values compared to that of perennial grass-legume mixtures (4358.33 kg/da – *Lolium perenne* L.+ *Trifolium pratense* L.; 4220.83 kg/da – *Lolium perenne* L. + *Trifolium*

repens L. and 4270.83 kg/da – *Lolium perenne* L. + *Lotus corniculatus* L.), where legume species are the main component in the grass stand.

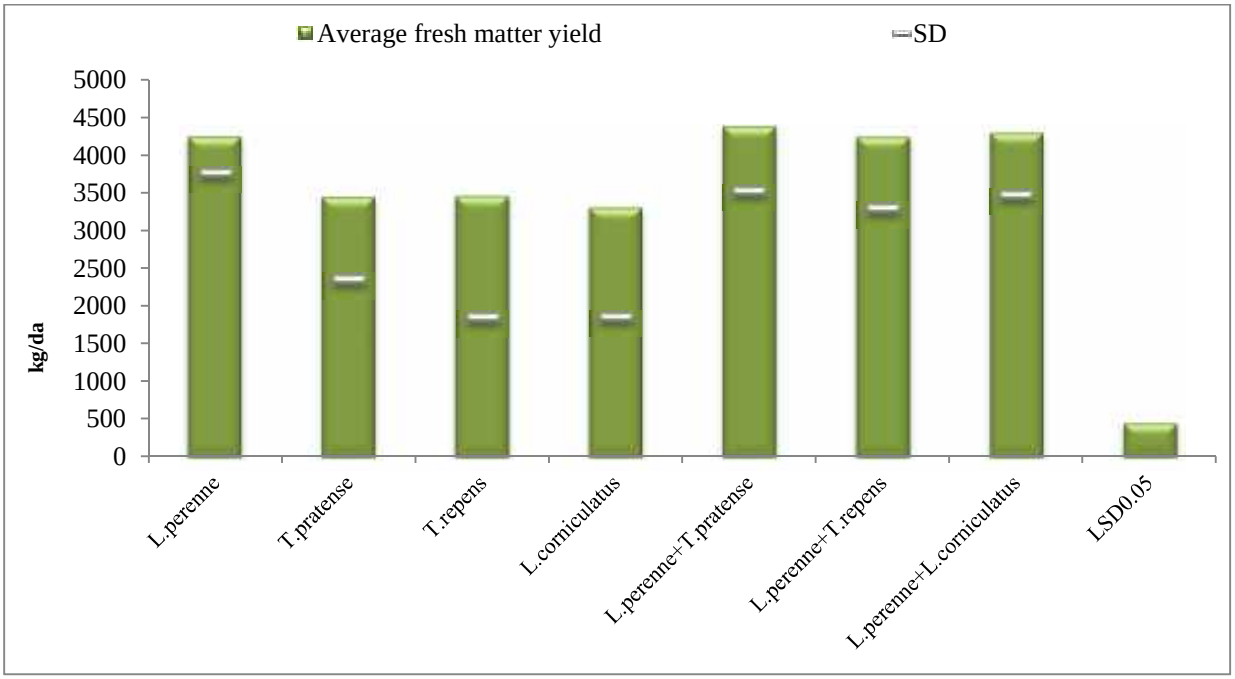


Figure 7. Yield of fresh matter from monoculture and mixed grass stands of perennial ryegrass and forage legumes, on average for the period 2020-2022

Over the three-year study period, the average yield of fresh mass from monocultures of perennial ryegrass was 4225.33 kg/da. The values are not significantly different from those of the mixed grass stands, where the grass crop is involved. A minimal excess in the yield of fresh mass was observed only in its mixture with white clover.

Yield of dry matter from monoculture and mixed grass stands of perennial ryegrass and forage legumes.

In the first year, the amount of dry matter of the monoculture grass stands of perennial ryegrass (542.25 kg/da) had a slight advantage over its mixtures with white clover (516.12 kg/da) and bird's-foot-trefoil (540.80 kg/da) – Table 11a.

In total for 2021, with the highest dry matter productivity is the mixture of perennial ryegrass – red clover (1810.42 kg/da), followed by white clover (1809.86 kg/da) and bird's-foot-trefoil (1772.06 kg /da). The analysis of the results indicates a significant advantage in the yield of dry mass in the mixed cultivation of white clover and bird's-foot-trefoil compared to their monoculture grass stands. The excess in the values of the indicator was by 34.50% and 37.80% (at P = 0.05), respectively, in the mixtures – *Lolium perenne* L. + *Trifolium repens* L. and *Lolium perenne* L. + *Lotus corniculatus* L. The yield of dry matter in the monoculture grass stands with legume crops varied from 1286.00 kg/da (*Lotus corniculatus* L.) to 1462.45 kg/da (*Trifolium pratense* L.). The dry matter yield of *Lolium perenne* L. is 1796.67 kg/da.

The yield of dry matter in the mixtures of perennial ryegrass with red and white clover is higher, compared to the amount of dry mass obtained in the monoculture grass stands of the grass and legume component. An exception is the mixture *Lolium perenne* L. + *Lotus corniculatus* L., where the monoculture grass stands of *Lolium perenne* L. have a 24.61 kg/da higher annual dry matter yield.

Table 11a. Yield of dry matter from monoculture and mixed grass stands of perennial ryegrass and forage legumes, by years (kg/da)

Variants	2020	2021			2022
		I regrowth	II regrowth	Total	
Lolium perenne L.	542.25	1550.34	246.33	1796.67	666.83
Trifolium pratense L.	410.25	973.39	489.07	1462.45	815.50
Trifolium repens L.	518.73	859.56	486.08	1345.64	1059.81
Lotus corniculatus L.	570.13	888.36	397.64	1286.00	959.91
Lolium perenne L.+ *Trifolium pratense* L.	575.57	1492.05	318.37	1810.42	899.77
Lolium perenne L. + *Trifolium repens* L.	516.12	1484.64	325.22	1809.86	1008.31
Lolium perenne L. + *Lotus corniculatus* L.	540.80	1472.83	299.23	1772.06	847.22
LSD$_{0.05}$	***98.35***	***326.13***	***153.38***	***441.56***	***254.36***
LSD$_{0.01}$	***133.90***	***444.02***	***208.83***	***601.18***	***346.30***
LSD$_{0.001}$	***180.62***	***598.95***	***281.69***	***810.94***	***467.13***

In the third year, white clover (1059.81 kg/da) had the highest yield of dry mass in the monoculture grass stands with legume crops. Its mixture with perennial ryegrass also had the highest value of the indicator (1008.31 kg/da) compared to the other two-component mixtures and with a significant advantage over the yield of dry mass in the monoculture cultivation of the grass crop (666.83 kg/da).

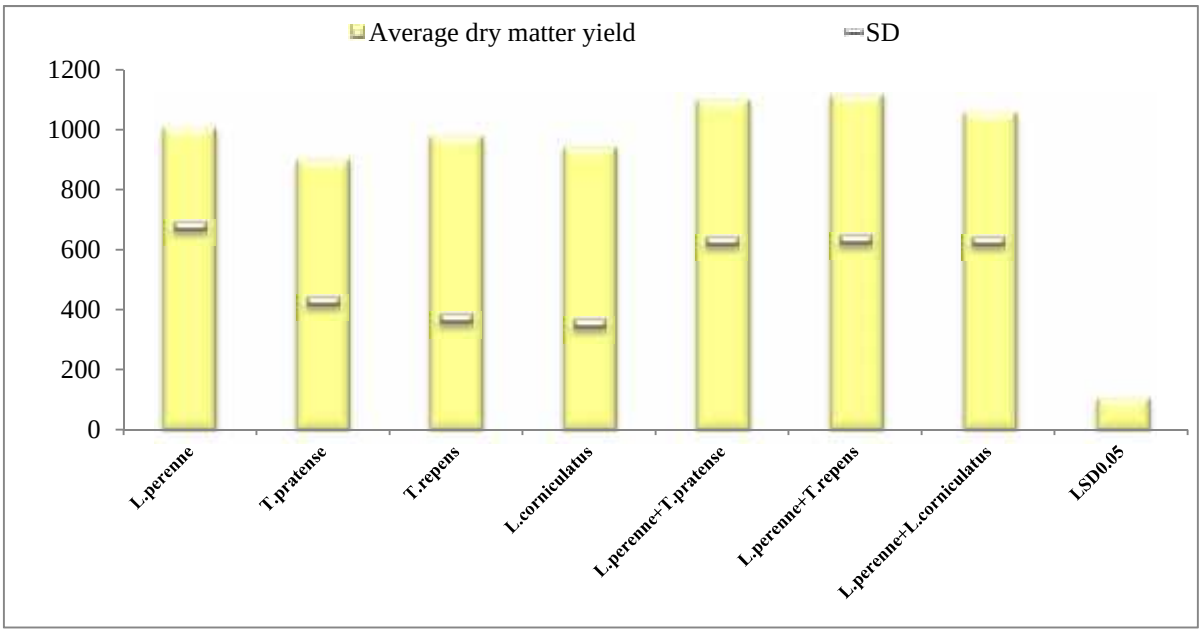


Figure 8. Yield of dry matter (kg/da) from monoculture and mixed grass stands of perennial ryegrass and forage legumes, average for the period 2020-2022

On average for the period (Figure 8), the yield of dry mass in the two-component mixtures was higher and with a proven difference compared to the amount of dry mass obtained from the monoculture grass stands of legume forage crops. The excess in the values of the indicator are respectively 12.22% (in the mixture *Lolium perenne* L. + *Lotus corniculatus* L.), 14.02% (in the mixture *Lolium perenne* L. + *Trifolium repens* L.) and 22.23% (in the mixture *Lolium perenne* L. + *Trifolium pratense* L.) at P <0.05.

Compared to perennial grass mixtures, the yield of dry mass in the monoculture cultivation of perennial ryegrass also registered lower values of the trait (1001.92 kg/da), but without a significant difference.

5.3.3. *Botanical composition of monoculture and mixed grass stands of perennial ryegrass and legume forage grasses.*

Botanical composition of monoculture grass stands of perennial ryegrass and legume forage grasses.

Weed vegetation was present at a high percentage in the grass stand with the monoculture crops of perennial ryegrass and legumes in the year of sowing (Figure 9).

For the second experimental year, the weed infestation of the monoculture grass stands with legume crops in the first cut ranged from 3.85% (*Trifolium pratense* L.) to 8.33% (*Lotus corniculatus* L.). The results of the analysis indicate that the main crop had a high presence in the grass stand, namely: red clover with 96.15%, white clover with 95.45% and bird's-foot-trefoil with 91.67%.

Lolium perenne L. also showed a high share percentage (98.55%) in the formed biomass and a low percentage of weed infestation (1.45%).

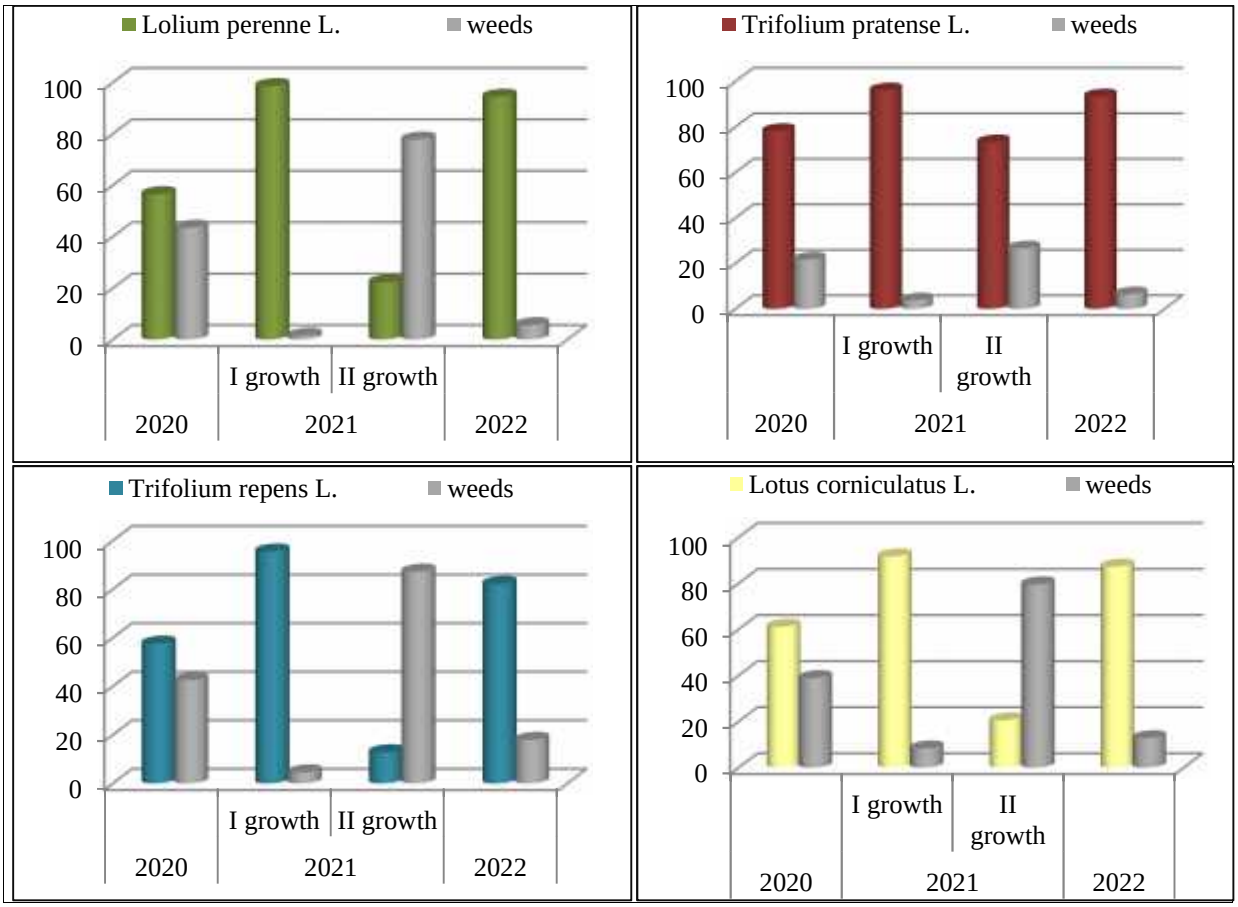


Figure 9. Botanical composition of monoculture grass stands with perennial ryegrass and legume forage grasses (by years)

In the second cut in 2021, the weed infestation rate was high in all tested variants of monoculture crops with perennial ryegrass and forage legumes.

In the third year, the highest degree of weed infestation was registered in the grass stands with white clover (legume:weed ratio of 82.10:17.90%), followed by bird's-foot-trefoil (legume:weed ratio of 87.20:12.80) and red clover (legume:weed ratio – 93.50:6.50%).

Perennial ryegrass as a monoculture had a high share (94.40%) in the above-ground mass compared to weed vegetation (5.60%).

Botanical composition of mixed grass stands of perennial ryegrass and legume fodder crops.

In the year of sowing, perennial ryegrass occupied the largest share in mixtures with bird's-foot-trefoil. The ratio of grass and legume crops was 56.00:20.00%. *Lolium perenne* L. was also a dominant component (with 41.45%) in the mixture with white clover (23.32%), where was the highest percentage of weeds (35.23%) – Figure 10. In the year of grass stands' establishment, the mixed crop of perennial ryegrass with red clover had the most balanced ratio of both components (grasses:legumes – 43.10:36.78%). The grass stands also had the lowest weed infestation (20.11%) compared to the mixtures of perennial ryegrass with bird's-foot-trefoil (24.00%) and white clover (35.23%).

In the first cut of the second experimental year (2021), the weed infestation of the crops in the three tested variants was relatively low. The perennial ryegrass-red clover mixture had an absolute balance of both components (47.62:47.62%) and the highest share of weed vegetation (4.76%) in the above-ground biomass.

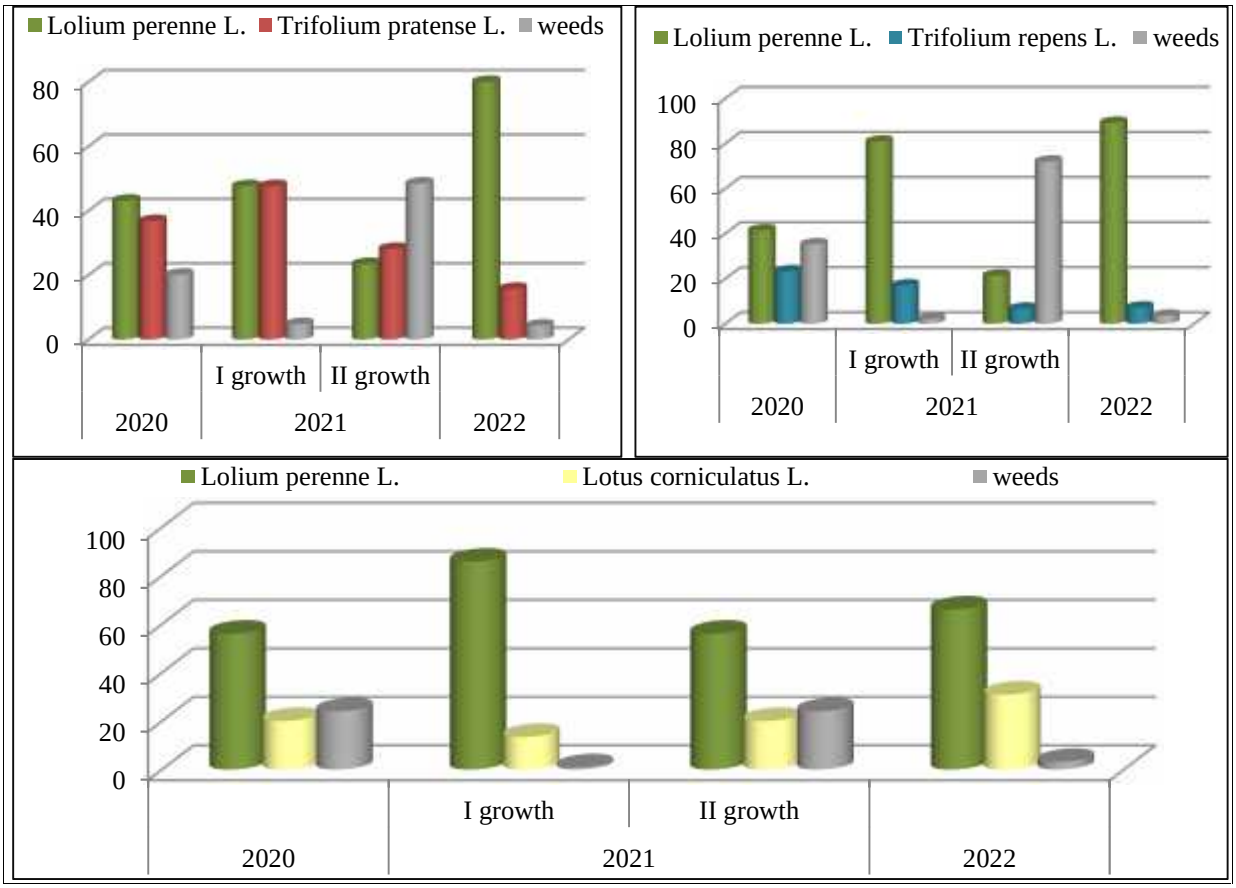


Figure 10. Botanical composition (%) of mixed grass stands of perennial ryegrass and forage legumes (by years)

In contrast, the second cut of the second vegetation marked significant weed infestation of the tested perennial grass-legume mixtures. The mixture of perennial ryegrass with red clover in this regrowth had also the most balanced percentage ratio of the tested components with 23.44:28.13% compared to the other two variants.

In the third experimental year, the weed infestation level in the mixed grass stands was relatively low. The values of the indicator varied from 3.10% (*Lolium perenne* L. + *Lotus corniculatus* L.) to 4.30% (*Lolium perenne* L. + *Trifolium pratense* L.). Perennial ryegrass dominated all three tested mixtures, as the highest share was found in the white clover grass

stands, where the grass:legume:weed ratio was 89.20:7.20:3.60%, respectively. Compared with the second year, where in the mixture with red clover the share of the components was almost equal, there was a significant decrease of the legume component in the third year. The share of the grass crop is 80.00%, and the legume crop – 15.70%.

In the third year, bird's-foot-trefoil marked the highest presence in the grass stand (30.90%) with perennial ryegrass, compared to the first and second experimental years. Compared to the other mixtures included in the experiment, the mixture was the least weedy (3.10%) and with a high participation of perennial ryegrass (66.00%).

The botanical analysis of the monoculture grass stands with legume fodder crops indicated that *Trifolium pratense* L. had the highest resistance and adaptability to the pre-mountain conditions of the Central Balkan Mountain. This variant had the lowest weed infestation degree and the highest share of the main crop in the formed biomass. This trend is also confirmed with the share of the legume component in the mixed grass stands. Compared to the other legume species during the experimental years, red clover retained relatively the highest share (over 32.06%) in mixtures with perennial ryegrass, followed by bird's-foot-trefoil (over 21.07%) and white clover (over 13.55%).

Lolium perenne L. grown as a monoculture, registered a high presence of the wheat component (67.92%) in the volume of the above-ground mass, and the share of weed vegetation was 32.08%. In the mixed grass stands, perennial ryegrass maintained the highest presence in the two-component mixture with bird's-foot-trefoil (65.93%), followed by the mixture with white (58.21%) and red clover (48.54%).

5.3.4. Main chemical composition of monoculture and mixed grass stands of perennial ryegrass and forage legumes.

The chemical composition of the fodder mass is a determining factor for the quality of the grass stands.

During the first vegetation, the perennial ryegrass prevails in the plant biomass compared to the legume representative, which is a prerequisite for the formation of biomass with a high carbohydrate content. In its mixture with red clover, the values of nitrogen-free extractable substances were the highest (382.10 g kg^{-1} DM) – Table 12.

In the second vegetation, monoculture grass stands with perennial ryegrass were lower in ash (5.41 to 17.91%) and phosphorus (2.12 to 17.99%), but richer in nitrogen-free extractable substances compared to the mixed ones. Associated with legume fodder crops, the crude protein in the dry matter of its two-component mixtures increased from 29.10% (*Lolium perenne* L. + *Trifolium repens* L.) to 47.97% (*Lolium perenne* L. + *Lotus corniculatus* L.), and the crude fiber content varied from 365.25 g kg^{-1} DM (*Lolium perenne* L. + *Lotus corniculatus* L.) to 486.15 g kg^{-1} DM (*Lolium perenne* L.+ *Trifolium pratense* L.).

In the third year, the participation of *Lolium perenne* L. in the grass stands with white clover was < 89.00%, which affects the crude protein content of the forage mass. The mixture compared to the others included in the experiment had the highest content of dry matter (915.60 g kg^{-1} DM), NFE (344.40 g kg^{-1} DM) and phosphorus (5.45 g kg^{-1} DM), but poorest in crude protein (121.80 g kg^{-1} DM).

The values of these indicators are completely opposite in the mixture of perennial ryegrass with red clover, where the amount of crude protein (167.30 g kg^{-1} DM) and was the highest, and that of nitrogen-free extractable substances (299.70 g kg^{-1} DM) and phosphorus (1.91 g kg^{-1} DM) – the lowest. In the *Lolium perenne* L.+ *Trifolium pratense* L. mixture, the following regularity was observed: the high crude protein content was combined with the highest crude fiber content (388.30 g kg^{-1} DM). This atypical manifestation is due to the botanical characteristics of the crops in the grass association, in this case the reaction of the legume crop to the grass crop and the regime (mowing) of grass stands use.

Table 12. Main chemical composition (g kg^{-1} DM) from monoculture and mixed grass stands of perennial ryegrass and forage legumes, over the years and average for the period 2020-2022.

Variants	DM	CP	CFr	CF	Ash	NFE	Ca	P
2020								
Lolium perenne L.	900.90	101.60	416.80	40.70	86.30	255.50	14.70	2.46
Trifolium pratense L.	894.10	181.50	220.60	24.90	75.50	391.60	17.00	0.98
Trifolium repens L.	890.70	214.10	197.80	29.70	83.60	365.50	26.00	2.15
Lotus corniculatus L.	899.40	196.20	338.90	30.50	60.80	273.00	14.70	0.68
L. perenne L.+*Tr. pratense* L.	894.00	139.80	257.10	31.30	83.70	382.10	12.50	1.95
L. perenne L.+*Tr. repens* L.	895.00	135.60	277.80	35.20	76.60	369.80	21.50	2.20
L. perenne L.+*L. corniculatus* L.	897.20	127.00	313.60	34.60	71.50	350.50	21.40	3.07
2021								
Lolium perenne L.	916.75	102.05	413.85	25.05	50.80	325.00	17.65	1.89
Trifolium pratense L.	911.05	168.15	302.75	44.85	69.75	325.55	17.75	2.45
Trifolium repens L.	916.60	217.25	349.80	26.40	70.35	252.80	16.55	3.19
Lotus corniculatus L.	925.95	194.40	413.25	35.95	59.15	223.20	15.30	2.89
L. perenne L.+*Tr. pratense* L.	920.65	147.40	486.15	22.30	59.90	204.90	12.15	2.17
L. perenne L.+*Tr. repens* L.	916.70	131.75	403.15	31.65	55.75	294.40	12.20	1.93
L. perenne L.+*L. corniculatus* L.	921.25	151.00	365.25	26.50	53.55	324.95	13.20	2.23
2022								
Lolium perenne L.	910.30	105.50	359.10	16.30	44.20	385.20	17.90	2.05
Trifolium pratense L.	907.10	192.90	381.40	19.30	39.00	274.50	21.20	3.36
Trifolium repens L.	911.10	201.40	259.80	26.50	37.20	386.20	21.10	2.62
Lotus corniculatus L.	911.90	186.90	278.40	23.60	45.20	377.80	10.10	3.25
L. perenne L.+*Tr. pratense* L.	913.30	167.30	388.30	11.30	46.70	299.70	14.50	1.91
L. perenne L.+*Tr. repens* L.	915.60	121.80	387.90	16.30	45.20	344.40	12.20	5.45
L. perenne L.+*L. corniculatus* L.	911.60	156.30	374.90	20.70	40.60	319.10	11.00	4.11
2020-2022								
Lolium perenne L.	909.32	103.05	396.58	27.35	60.43	321.90	16.75	2.13
Trifolium pratense L.	904.08	180.85	301.58	29.68	61.42	330.55	18.65	2.26
Trifolium repens L.	906.13	210.92	269.13	20.87	63.72	334.83	21.22	2.65
Lotus corniculatus L.	912.42	192.50	343.52	30.02	55.05	291.33	13.37	2.27
L. perenne L.+*Tr. pratense* L.	909.32	151.50	377.18	21.63	63.43	295.57	13.05	2.01
L. perenne L.+*Tr. repens* L.	909.10	129.72	356.28	27.72	59.18	336.20	15.30	3.19
L. perenne L.+*L. corniculatus* L.	910.02	144.77	351.25	27.27	55.22	331.52	15.20	3.14
Average	***908.63***	***159.04***	***342.22***	***26.36***	***59.78***	***320.27***	***16.22***	***2.52***
MAX	***912.42***	***210.92***	***396.58***	***30.02***	***63.72***	***336.20***	***21.22***	***3.19***
MIN	***904.08***	***103.05***	***269.13***	***20.87***	***55.05***	***291.33***	***13.05***	***2.01***
Significance of the differences in the values of the indicator over the years	P > 0.05	P < 0.001	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
Significance of differences in indicator values among variants	**P < 0.001**	P > 0.05	**P = 0.01**	**P < 0.01**	**P < 0.001**	P > 0.05	P > 0.05	P > 0.05

The amount of mineral substances in the mixed grass stands varied from 40.60 g kg^{-1} DM (*Lolium perenne* L. + *Lotus corniculatus* L.) to 46.70 g kg^{-1} DM (*Lolium perenne* L.+ *Trifolium pratense* L.).

Averaged over the period, white clover in monoculture grass stands with legume fodder crops had the highest dry matter crude protein content (210.92 g kg^{-1}). The difference in the values of the indicator in comparison with other legumes is reliable (P <0.001) and highly dependent on the conditions of the year. In the two-component mixtures, the biomass of perennial ryegrass with red clover had the highest protein content was (151.50 g kg^{-1} DM) followed by its mixture with bird's-foot-trefoil (144.77 g kg^{-1} DM) and white clover (129.72 g kg^{-1} DM).

The difference in the crude fiber content of the studied monoculture and mixed grass stands of perennial ryegrass and legume fodder crops was statistically significant (P = 0.01). The lowest fibre content (269.13 g kg^{-1} DM) was found in the dry mass of white clover from the group of legumes. The decrease was 21.36% compared to the average value of the indicator. In the mixed grass stands, the amount of crude fiber was 2.64% higher (*Lolium perenne* L. + *Lotus corniculatus* L.) to 10.22% (*Lolium perenne* L. + *Trifolium pratense* L.) compared to the average (342.22 g kg^{-1} DM).

It was established that the cultivation method and the composition of the grass stand are factors that have a significant impact (P <0.001) on the concentration of dry matter and mineral substances in the investigated grass stands. On average for the period, the monoculture grass stands of *Lotus corniculatus* L. (912.42 g kg^{-1} DM) and its mixtures with *Lolium perenne* L. (910.02 g kg^{-1} DM) had the highest dry matter content. White clover (63.72 g kg^{-1} DM) and the mixture of perennial ryegrass with red clover (63.43 g kg^{-1} DM) recorded the highest ash content.

5.3.5. *Structural fiber components of cell walls of monoculture and mixed grass stands of perennial ryegrass and legume forage grasses.*

The fiber structural components of cell walls are an important factor in ruminant feeding and productivity.

In the year of sowing, the mixed crop of *Lolium perenne* L. with *Lotus corniculatus* L. formed a grass stand with maximum values of NDF (531.50 g kg^{-1} DM), ADL (296.30 g kg^{-1} CB), ADF (64.30 g kg^{-1} DM) and cellulose (232.00 g kg^{-1} DM) compared to the rest of the mixed grass stands (Table 13). What is interesting in this case is that the grass component had a positive effect on the amount of polyoside – hemicellulose, which is fully digestible by animals. In the monoculture grass stands the values of the indicator of the legume are the lowest (103.40 g kg^{-1} DM) and the highest (235.20 g kg^{-1} DM) in the mixed grass stands of the legume crop.

In the second year in the regrowths of perennial grass and legume mixtures, the dominant component is perennial ryegrass. The most fibrous (NDF – 640.60 g kg^{-1} DM, ADF – 383.90 g kg^{-1} DM, hemicellulose – 256.70 g kg^{-1} CB and cellulose – 329.85 g kg^{-1} DM) and with the lowest digestibility of dry matter (623.56 g kg^{-1} DM) is the grass mass of *Lolium perenne* L. + *Trifolium repens* L. In contrast to the first vegetation, the grass stands of *Lolium perenne* L. + *Lotus corniculatus* L. have the lowest content of ADF (360.20 g kg^{-1} DM) and, accordingly, with the highest *in vitro* digestibility of dry matter (630.46 g kg^{-1} DM).

Table 13. Fiber structural components of cell walls and *in vitro* digestibility (g kg^{-1} DM) of dry matter of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands over the years and average for the period 2020-2022

Variants	NDF	ADF	ADL	Hemicellulose	Cellulose	DMD according to Aufrere
2020						
Lolium perenne L.	558.70	330.60	93.30	228.10	237.30	645.87
Trifolium pratense L.	394.60	220.20	46.10	174.40	174.10	692.71
Trifolium repens L.	339.10	210.70	57.40	128.40	153.30	713.41
Lotus corniculatus L.	422.40	319.00	81.30	103.40	237.70	660.85
L. perenne L.+*Tr. pratense* L.	445.50	272.60	59.20	172.90	213.40	634.18
L. perenne L.+*Tr. repens* L.	477.70	254.50	39.90	223.20	214.60	651.23
L. perenne L.+*L. corniculatus* L.	531.50	296.30	64.30	235.20	232.00	635.08
2021						
Lolium perenne L.	656.95	372.75	88.90	284.20	283.85	650.42
Trifolium pratense L.	402.50	257.20	64.00	145.30	193.20	662.65
Trifolium repens L.	387.30	280.90	55.50	106.40	225.40	708.80
Lotus corniculatus L.	434.85	309.95	62.85	124.90	247.10	690.91
L. perenne L.+*Tr. pratense* L.	569.60	368.55	77.10	201.05	291.45	628.11
L. perenne L.+*Tr. repens* L.	640.60	383.90	54.05	256.70	329.85	623.56
L. perenne L.+*L. corniculatus* L.	598.00	360.20	65.20	237.80	295.00	630.46
2022						
Lolium perenne L.	646.80	392.80	81.20	254.00	311.60	620.53
Trifolium pratense L.	457.00	259.50	69.80	197.50	189.70	644.48
Trifolium repens L.	329.80	220.90	53.10	108.90	167.80	745.68
Lotus corniculatus L.	358.20	247.00	68.00	111.20	179.00	697.15
L. perenne L.+*Tr. pratense* L.	483.30	303.20	66.20	180.10	237.00	604.24
L. perenne L.+*Tr. repens* L.	427.30	257.10	52.00	170.20	205.10	652.84
L. perenne L.+*L. corniculatus* L.	457.00	273.80	64.90	183.20	208.90	643.31
2020-2022						
Lolium perenne L.	620.82	365.38	87.80	255.43	277.58	638.94
Trifolium pratense L.	418.03	245.63	59.97	172.40	185.67	666.61
Trifolium repens L.	352.07	237.50	55.33	114.57	182.17	722.63
Lotus corniculatus L.	405.15	291.98	70.72	113.17	221.27	682.97
L. perenne L.+*Tr. pratense* L.	499.47	314.78	67.50	184.68	247.28	622.18
L. perenne L.+*Tr. repens* L.	515.20	298.50	48.65	216.70	249.85	642.54
L. perenne L.+*L. corniculatus* L.	528.83	310.10	64.80	218.73	245.30	636.28
Average	***477.08***	***294.84***	***64.97***	***182.24***	***229.87***	***658.88***
MAX	***620.82***	***365.38***	***87.80***	***255.43***	***277.58***	***722.63***
MIN	***352.07***	***237.50***	***48.65***	***113.17***	***182.17***	***622.18***
Significance of the differences in the values of the indicator over the years	**P < 0.001**	**P < 0.01**	**P < 0.01**	**P < 0.001**	**P = 0.01**	**P < 0.001**
Significance of differences in indicator values between variants	**P < 0.05**	**P < 0.01**	P > 0.05	P > 0.05	**P < 0.01**	**P < 0.05**

As the age of the grass stand progresses (in the third vegetation), the biomass of the white clover grown as monoculture and in two-component mixtures has the best quality characteristics. In monoculture crops, the species recorded the lowest concentrations of fiber structural components of cell walls and the highest *in vitro* digestibility of dry matter. This regularity is also preserved in mixtures with perennial ryegrass. In contrast, the monoculture and mixed grass stands of red clover registered maximum values of the investigated fiber fractions, respectively the lowest in vitro digestibility of dry matter. Over the experimental period, monoculture grass stands of perennial ryegrass had a higher degree of lignification

(coefficient = 14.14) compared to its mixtures with the three subspecies of legume fodder crops (Figure 11). On average, the excess was from 4.66% (*Lolium perenne* L.+ *Trifolium pratense* L.) to 49.79% (*Lolium perenne* L. + *Trifolium repens* L.). The coefficients determining the degree of lignification in the fodder biomass of the monoculture grass stands in legumes are higher and vary from 14.34 (*Trifolium pratense* L.) to 17.45 (*Lotus corniculatus* L.).

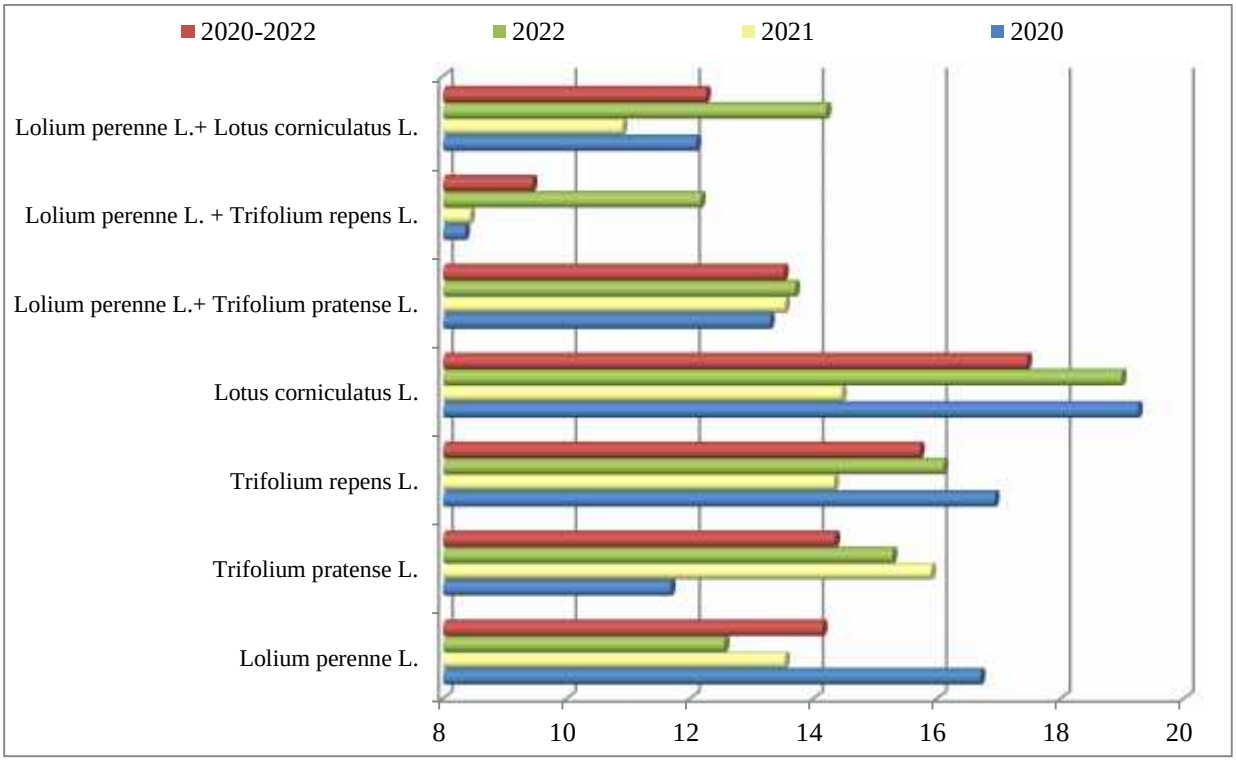


Figure 11. Lignification degree of forage of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands, by year and average for the period (coefficient)

The concentration of fiber fractions is essential for fodder quality.

Averaged over the period, the sources of variation (years and variants) had a significant effect on the amount of NDF ($P < 0.001$ and $P < 0.05$), ADF ($P < 0.01$) and cellulose ($P = 0.01$ and $P < 0.01$). Environmental conditions have been proven to affect the content of acid detergent lignin ($P < 0.01$) and hemicellulose ($P < 0.001$) in the dry matter of the studied variants.

It was found that the concentration of the fiber components of the cell walls in the monoculture grass stands of perennial ryegrass was significantly higher compared to the mixed ones. The decrease in the values of the indicators in the two-component mixtures compared to that in the monoculture cultivation of *Lolium perenne* L. varies:

- from 14.82% (*Lolium perenne* L. + *Lotus corniculatus* L.) to 19.55% (*Lolium perenne* L.+ *Trifolium pratense* L.) for neutral detergent fibers;
- from 13.85% (*Lolium perenne* L.+ *Trifolium pratense* L.) to 18.30% (*Lolium perenne* L. + *Trifolium repens* L.) for acid detergent fiber;
- from 23.12% (*Lolium perenne* L.+ *Trifolium pratense* L.) to 44.59% (*Lolium perenne* L. + *Trifolium repens* L.) for acid detergent lignin;
- from 14.37% (*Lolium perenne* L. + *Lotus corniculatus* L.) to 27.70% (*Lolium perenne* L.+ *Trifolium pratense* L.) for hemicellulose and
- from 9.99% (*Lolium perenne* L. + *Trifolium repens* L.) to 11.63% (*Lolium perenne* L. + *Lotus corniculatus* L.) for cellulose.

The fiber composition of the cell walls is also a determining factor for fodder mass digestibility. It was established that the component composition of the grass mass ($P < 0.05$) and the conditions in the cultivation area ($P < 0.001$) had a proven impact on the values of the indicator. The biomass of the mixture *Lolium perenne* L. + *Trifolium repens* L. (642.54 g kg^{-1} DM) had the highest dry matter digestibility, followed by the mixtures of *Lolium perenne* L. with *Lotus corniculatus* L. (636.28 g kg^{-1} DM) and *Lolium perenne* L. with *Trifolium pratense* L. (622.18 g kg^{-1} DM).

5.3.6. *Potential energy nutritive value of fodder from monoculture and mixed grass stands of perennial ryegrass legume fodder crops.*

In Table 14, an assessment of the energy nutritional value of fodder in monoculture and mixed grass stands of perennial ryegrass and forage legumes is made.

In the first year, in the mixed crop of *Lolium perenne* L. with *Lotus corniculatus* L. the gross energy values were the highest (16.85 MJ/kg DM) compared to the other two-component grass stands, but lower compared to the monoculture grass stands with legumes.

In the monoculture and mixed grass stands with legumes, red clover and its mixture with perennial ryegrass had the lowest gross energy values (16.57 and 16.40 MJ/kg DM, respectively).

The biomass from the variants with perennial ryegrass (in monoculture crop) has the lowest exchangeable (6.69 MJ/kg DM) and net energy nutritional value – feed units for milk (0.60 units in kg DM) and feed units for growth (0.53 units in kg DM).

In the second year, the results obtained are unidirectional with those in the first year. The monoculture grass stands with bird's-foot-trefoil again have the highest energy (18.45 MJ/kg DM), whereas the biomass from mixtures with ryegrass respectively with the least amount of gross energy (17.78 MJ/kg DM), but with the highest content of EE (7.52 MJ/kg DM), FUM (0.69 units in kg DM) and FUG (0.62 units in kg DM).

Trifolium pratense L. grown as a monoculture form grass stands in with the lowest gross energy (17.57 MJ/kg DM) and the highest exchangeable (7.64 MJ/kg DM) and net energy (FUM – 0.70 units in kg DM and FUG – 0.64 units in kg DM) energy, and the biomass from the two-component mixtures with ryegrass registered the highest values of total energy (18.03 MJ/kg DM) and the lowest values of exchangeable energy (6.79 MJ/kg DM), FUM (0.61 units in kg DM) and FUG (0.53 units in kg DM).

As the age of the grass stand increases, the nutritional value of the forage changes. In the third year, the above-ground mass in monoculture grass stands of red clover had the highest gross energy (18.01 MJ/kg DM), and that of bird's-foot-trefoil had the lowest (17.63 MJ/kg DM). Red clover also has the lowest content of exchangeable (7.44 MJ/kg DM) and net energy (FUM – 0.68 units in kg DM and FUG – 0.61 units in kg DM), whereas white clover respectively is with the highest values of these indicators (EE – 8.18 MJ/kg DM; FUM – 0.76 units in kg DM and FUG – 0.70 units in kg DM).

The total energy value in perennial ryegrass grass stands (grown as a monoculture) is from 1.80% (*Lolium perenne* L. + *Trifolium repens* L.) to 3.49% (*Lolium perenne* L. + *Lotus corniculatus* L.) lower in favor of its two-component mixtures, but with a higher content of feed units for milk and growth. The excess in the values of the indicators was respectively 1.47-2.99% (for FUM) and 1.64-3.33% (for FUG). In the mixtures with bird's-foot-trefoil, the established grass stands had the highest amount of exchangeable energy (7.49 MJ/kg DM).

Averaged over the period, monoculture grass stands with bird's-foot-trefoil had the highest gross energy content in the fodder (17.89 MJ/kg DM). A significant difference in the values of the indicator was found between the studied variants at a high degree of reliability ($P < 0.001$). The excess compared to the average value of the indicator is 2.76%. White clover

biomass had the lowest concentration (17.18 MJ/kg DM), but the highest content of exchangeable energy, FUM and FUG.

Table 14. Nutritional energy value of forage from monoculture and mixed grass stands of perennial ryegrass and legume forage crops, by year and average for the period 2020-2022.

Variants	GE	EE	FUM	FUG
2020				
Lolium perenne L.	17.00	6.69	0.60	0.53
Trifolium pratense L.	16.57	7.78	0.73	0.68
Trifolium repens L.	16.62	7.81	0.73	0.68
Lotus corniculatus L.	17.59	7.39	0.68	0.61
L. perenne L.+*Tr. pratense* L.	16.40	7.49	0.69	0.64
L. perenne L.+*Tr. repens* L.	16.66	7.47	0.69	0.63
L. perenne L.+*L. corniculatus* L.	16.85	7.35	0.68	0.61
2021				
Lolium perenne L.	17.56	7.20	0.66	0.58
Trifolium pratense L.	17.57	7.64	0.70	0.64
Trifolium repens L.	17.83	7.42	0.68	0.61
Lotus corniculatus L.	18.45	7.32	0.66	0.58
L. perenne L.+*Tr. pratense* L.	18.03	6.79	0.61	0.53
L. perenne L.+*Tr. repens* L.	17.79	7.25	0.66	0.59
L. perenne L.+*L. corniculatus* L.	17.78	7.52	0.69	0.62
2022				
Lolium perenne L.	17.20	7.47	0.69	0.62
Trifolium pratense L.	18.01	7.44	0.68	0.61
Trifolium repens L.	17.84	8.18	0.76	0.70
Lotus corniculatus L.	17.63	7.98	0.74	0.68
L. perenne L.+*Tr. pratense* L.	17.67	7.35	0.67	0.60
L. perenne L.+*Tr. repens* L.	17.51	7.37	0.67	0.61
L. perenne L.+*L. corniculatus* L.	17.80	7.49	0.68	0.61
2020-2022				
Lolium perenne L.	17.26	7.12	0.65	0.58
Trifolium pratense L.	17.39	7.62	0.70	0.64
Trifolium repens L.	17.18	7.71	0.71	0.65
Lotus corniculatus L.	17.89	7.57	0.69	0.62
L. perenne L.+*Tr. pratense* L.	17.37	7.21	0.66	0.59
L. perenne L.+*Tr. repens* L.	17.32	7.36	0.67	0.61
L. perenne L.+*L. corniculatus* L.	17.48	7.45	0.68	0.62
Average	***17.41***	***7.43***	***0.68***	***0.62***
MAX	***17.89***	***7.71***	***0.71***	***0.65***
MIN	***17.18***	***7.12***	***0.65***	***0.58***
Significance of the differences in the values of the indicator over the years	P > 0.05	P > 0.05	P > 0.05	P > 0.05
Significance of differences in indicator values between variants	**P < 0.001**	P > 0.05	P > 0.05	P > 0.05

GE – gross energy – MJ/kg DM; EE – exchange energy – MJ/kg DM; FUM – feed units for milk – units in kg of dry matter; FUG – feed units for growth – units in kg of dry matter.

The fodder mass of perennial ryegrass registered lower values for all the investigated parameters compared to its mixtures with legume fodder crops. The highest caloric content is the harvested grass stands of *Lolium perenne* L. with *Lotus corniculatus* L. (17.48 MJ/kg DM). Biomass also has the highest average value of energy useful for the normal course of physiological needs in the animal organism (7.45 MJ/kg DM), as well as in terms of the number of feed units for milk (0.68 units in kg DM) and growth (0.62 units in kg DM).

The mixture of the grass crop with white clover has the lowest total energy nutritional value (17.32 MJ/kg DM), and the one with red clover, registered the lowest values of EE (7.21 MJ/kg DM), FUM (0.66 units in kg DM) and FUG (0.59 units in kg DM).

5.3.7. *Correlation and regression dependences between quantitative and qualitative parameters of fodder from monoculture and mixed grass stands of perennial ryegrass and forage legumes.*

The correlation relationship (positive or negative) between the studied indicators proves the statistical dependence between them.

Data analysis shows that the yield of fresh mass in the monoculture and mixed grass stands of perennial ryegrass and legume fodder crops is in a strong positive correlation with the amount of neutral detergent fiber (r = 0.74) and hemicellulose (r = 0.80) – Table 15. The theoretical regression lines and the equations of regression dependence between the indicators are: indicated on Figures 12-18, where:

- y = 0.1766x - 166.1 with coefficient of determination – R^2 = 0.5535 (P <0.05) – Figure 12;

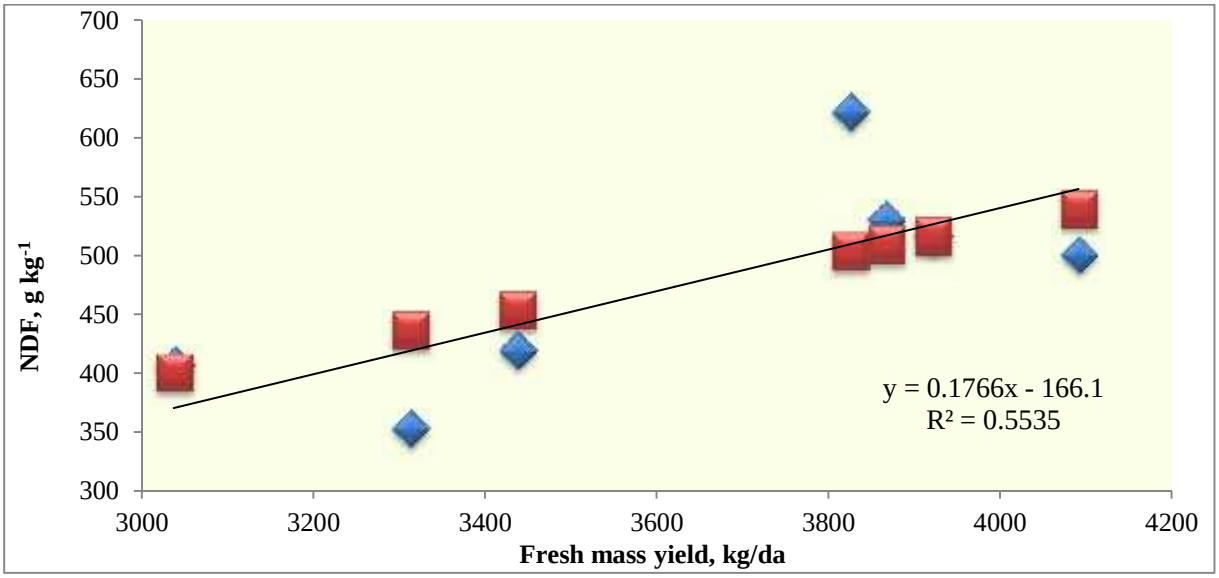


Figure 12. Regression dependence between the yield of fresh matter and the content of neutral detergent fibers in the dry matter of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

- y = 0.1118x - 224.95 with coefficient of determination – R^2 = 0.6347 (P <0.05) – Figure 13.

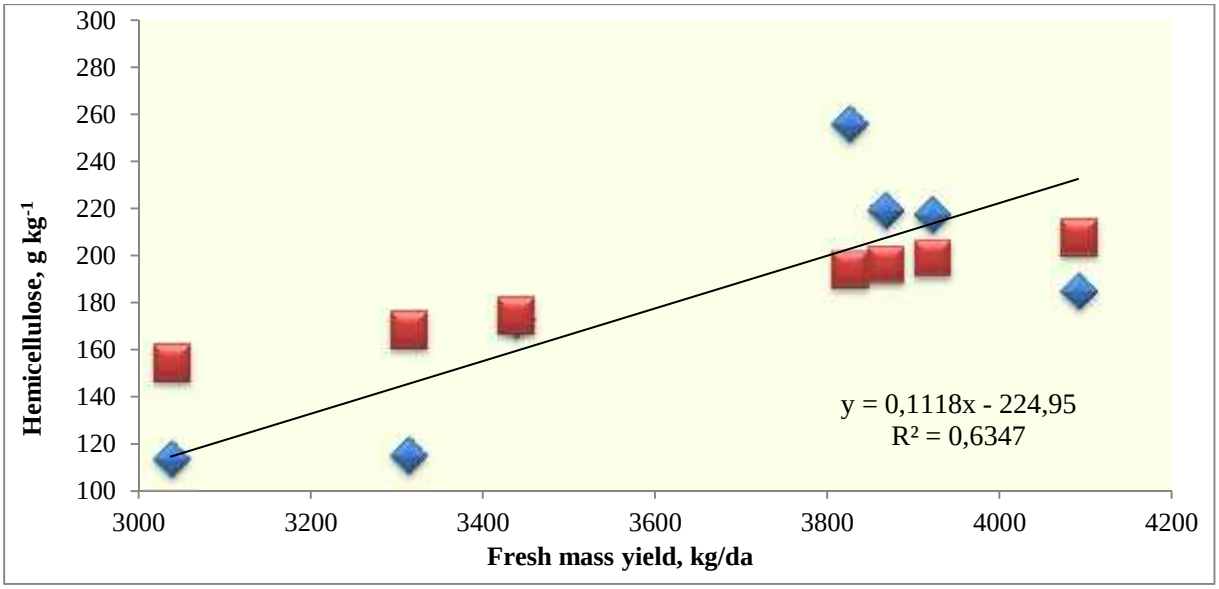


Figure 13. Regression dependence between the yield of fresh matter and the content of hemicellulose in the dry matter of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

In contrast, the relationship of the quantitative measure with crude protein content (r = -0.77), feed units for milk (r = -0.74) and growth (r = -0.70) and dry matter digestibility (r = -0.84) is negative.

The results of the correlation analysis (at a confidence level of 95%) show a statistically significant influence of the amount of crude protein on the *in vitro* digestibility of dry matter (r = 0.82) and the number of feed units (r = 0.88 – for FUM and r = 0.87 – for FUG) in the fodder mass. The developed regression models (Figure 14) are statistically proven at P <0.05 and allow to be used for tentative prediction of the feed units of biomass from monoculture and mixed grass stands of perennial ryegrass legume fodder crops.

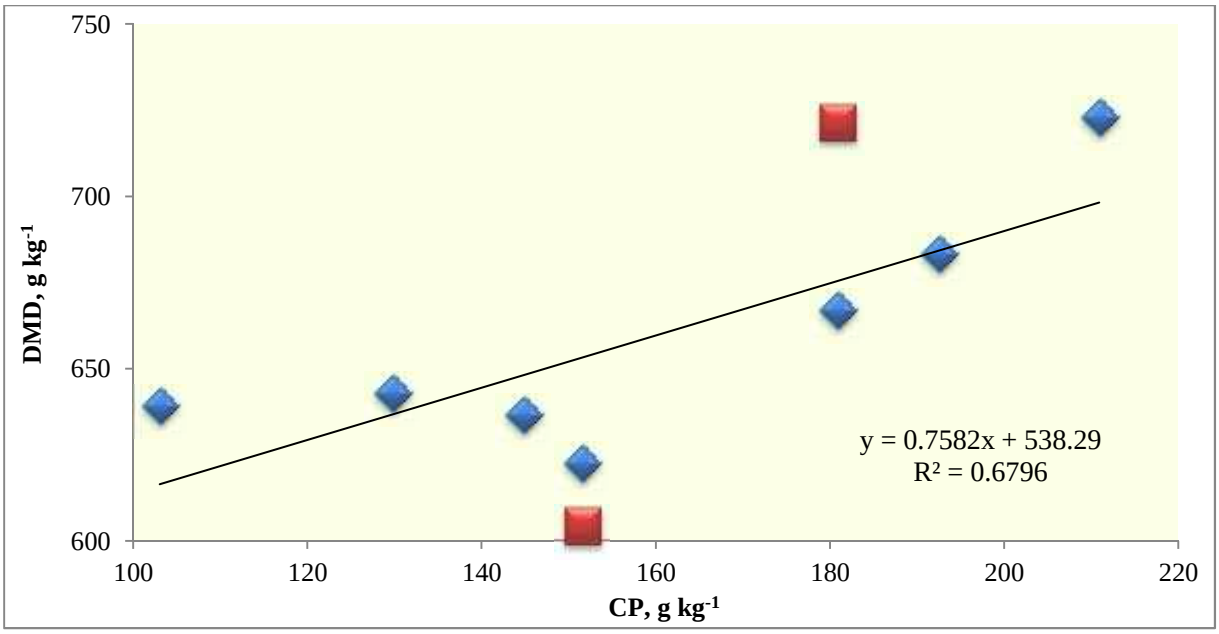


Figure 14. Regression dependence between crude protein content and *in vitro* dry matter digestibility of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

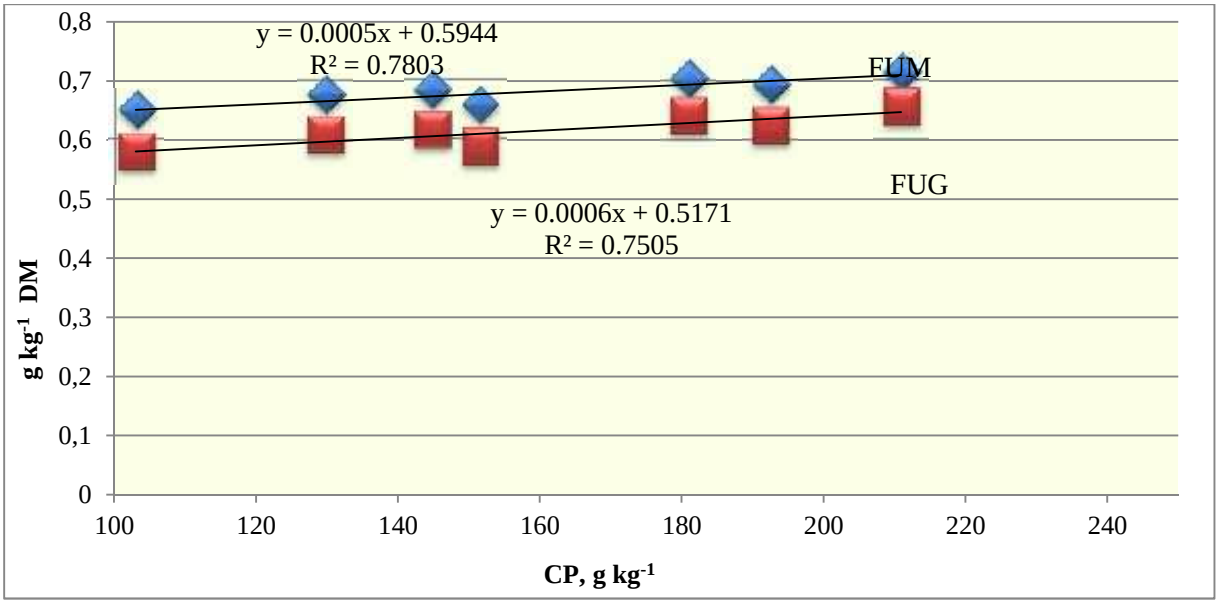


Figure 15. Regression dependence between the content of crude protein and the amount of feed units in the dry matter of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

A strong negative correlation dependence was found between the concentration of crude protein (as the main quality indicator in the composition of the dry matter) with the content of (Figure 15):

- Crude fibers (r = -0.86);
- Neutral detergent fibers (r = -0.98);
- Acid detergent fiber (r = -0.87);
- Hemicellulose (r = -0.96);
- Cellulose (r = -0.92)

and a strong positive correlation dependence between the amount of crude fiber and the structural fiber components of the cell walls (Figure 16), namely:

- Neutral detergent fibers (r = 0.88);
- Acid detergent fiber (r = 0.96);
- Hemicellulose (r = 0.72);
- Cellulose (r = 0.96)

The coefficients of determination between structural fiber components and crude fiber concentration ranged from R^2 = 0.5176 to R^2 = 0.9186, with very well-proven statistical significance of the equations. A high negative correlation also exists between crude fiber content with *in vitro* digestibility of dry matter (r = -0.85) and feed value - the number of feed units for milk (r = -0.96) and feed units for growth (r = -0.98) in dry matter. These indicators determine the nutritional value of fodder and allow its determination with relatively high accuracy, through the content of crude fiber.

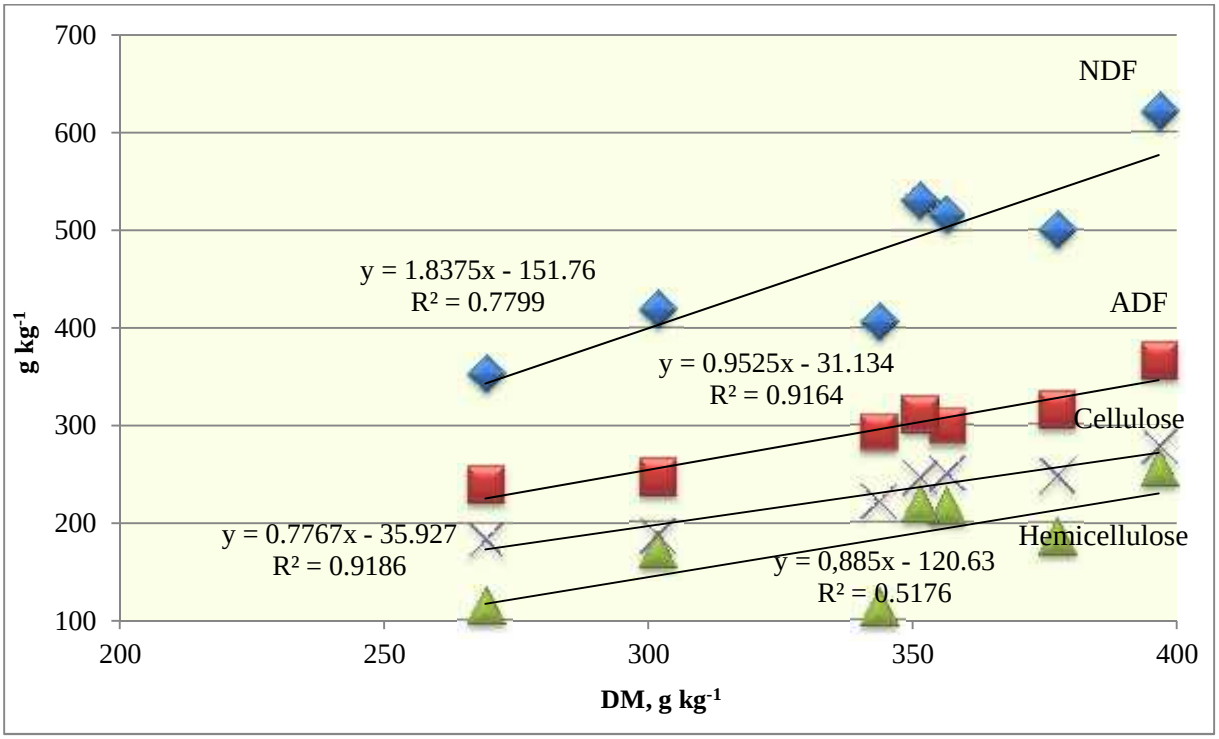


Figure 16. Regression dependence between the content of dry matter and the amount of NDF, ADF, cellulose and hemicellulose of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

The correlation coefficients of neutral detergent fibers with acid detergent fibers (r = 0.92), hemicellulose (r = 0.95) and cellulose (r = 0.93), as well as those of acid detergent

fibers with acid detergent lignin (r = 0.73), hemicellulose (r = 0.75) and cellulose (r = 0.97) were absolute values that corresponded to strong linear dependencies (Figures 17 and 18).

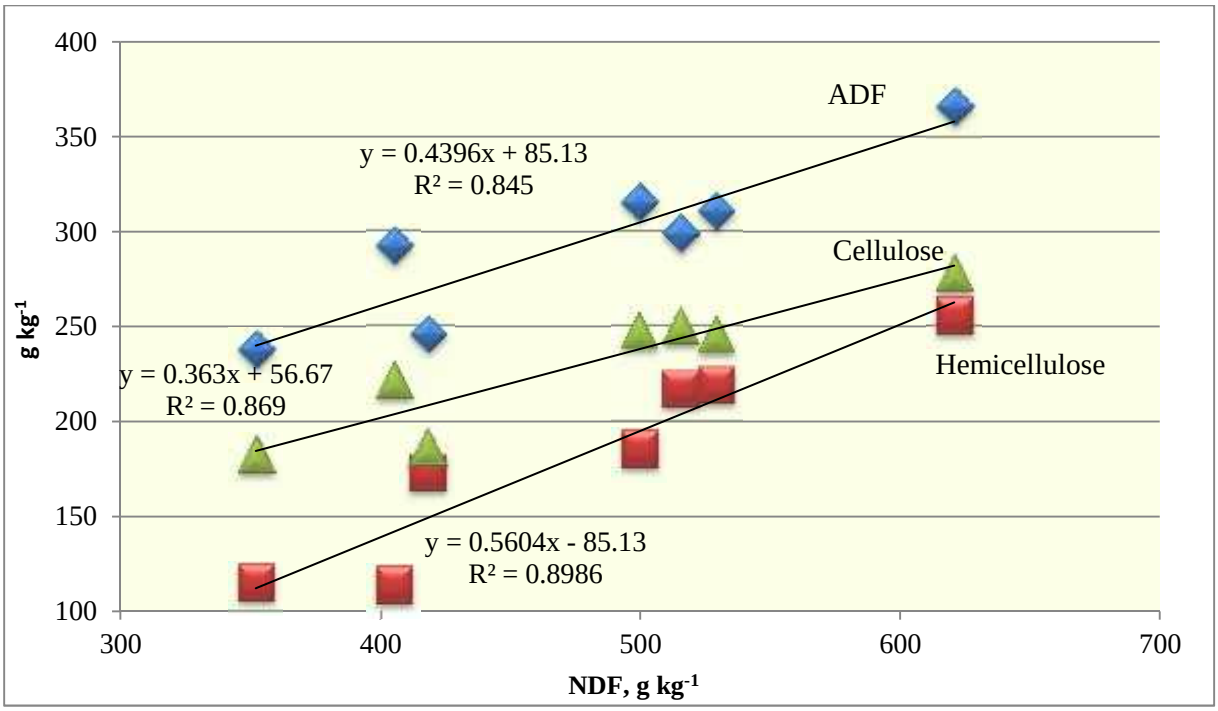


Figure 17. Regression dependence between the content of NDF with the amount of ADF, cellulose and hemicellulose in dry matter of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

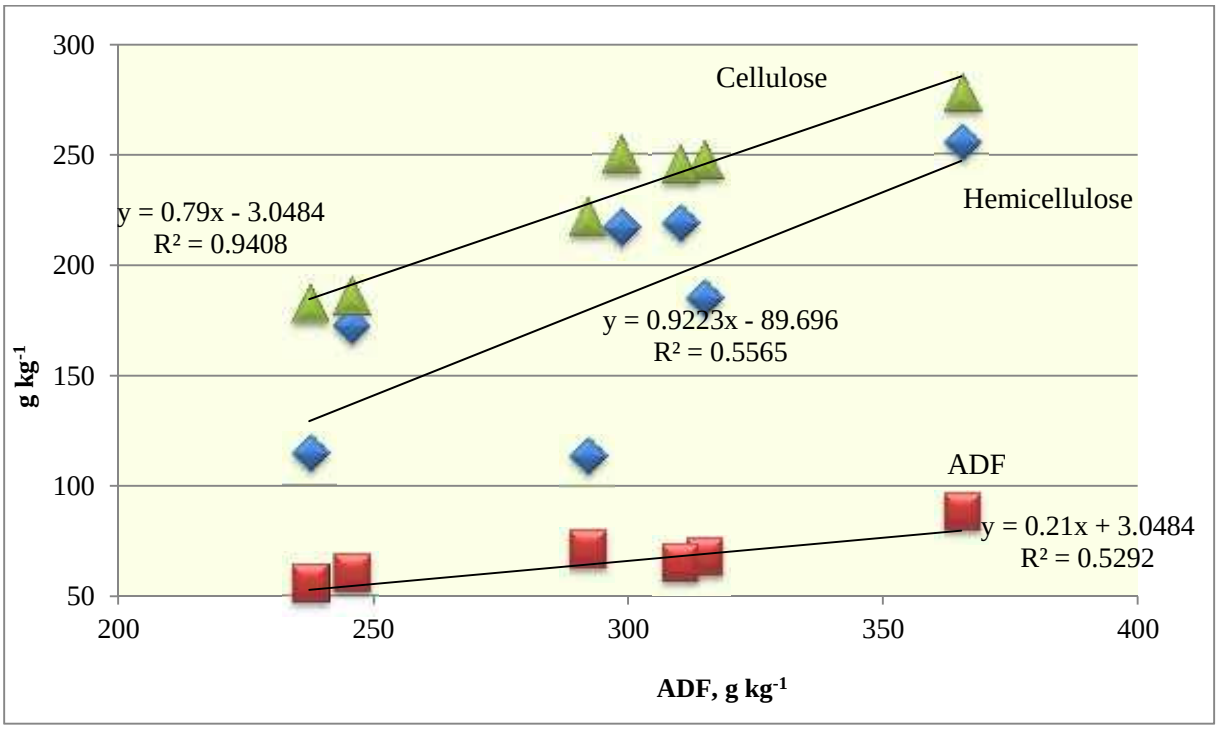


Figure 18. Regression dependence between the content of ADF and the amount of ADL, cellulose and hemicellulose in dry matter of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

Proving a high correlation and dependence between the fiber components of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands is essential to assess fodder quality.

Dry matter digestibility showed a positive correlation with feed units for milk and growth ($r = 0.84$) and strongly negative with fresh matter yield ($r = -0.84$), crude fiber ($r = -0.85$), neutral detergent fibers ($r = -0.83$), acid detergent fibers ($r = -0.73$), hemicellulose ($r = -0.80$) and cellulose ($r = -0.79$).

Negative correlation dependences were obtained for FUM and FUG with NDF, ADF, lignin and cellulose. This indicates that for predicting the energy nutritional value of fodder it is better to use crude fiber content as an independent variable. The reason is that in grass mixtures, grass and legume crops are characterized by a different morphological structure and the variation in crude fiber content is less compared to that of structural fiber components. On the other hand, it is much easier (and economically advantageous) for the practice to determine the feed value by the amount of crude fiber.

No statistically significant relationships were observed regarding the weight percentage of grass and legume crops in the composition of the grass stand with the monitored quantitative and qualitative parameters.

Table 15. Correlational dependences between indicators of composition, nutritional value and digestibility of perennial ryegrass and legume fodder crops in monoculture and mixed grasslands

	Fresh matter yield, kg/da	Dry matter yield, kg/da	Weight % grasses	Weight % legumes	CP g kg^{-1}	CFr g kg^{-1}	NDF g kg^{-1}	ADF g kg^{-1}	ADL g kg^{-1}	Hemicellulose g kg^{-1}	Cellulose g kg^{-1}	DMD g kg^{-1}	FUM	FUG
Fresh matter yield	1													
Dry matter yield	**0.83**	1												
grasses	0.20	0.36	1											
legumes	-0.32	-0.61	-0.14	1										
CP	-0.77	-0.58	-0.22	-0.01	1									
CFr	0.63	0.54	0.44	-0.22	-0.86	1								
NDF	**0.74**	0.53	0.17	0.04	-0.98	**0.88**	1							
ADF	0.57	0.48	0.36	-0.02	-0.87	**0.96**	**0.92**	1						
ADL	0.05	-0.19	0.19	0.43	-0.43	0.61	0.55	**0.73**	1					
Hemicellulose	**0.80**	0.51	-0.01	0.08	-0.96	**0.72**	**0.95**	**0.75**	0.35	1				
Cellulose	0.68	0.65	0.37	-0.17	-0.92	**0.96**	**0.93**	**0.97**	0.54	0.79	1			
DMD	-0.84	-0.62	-0.18	0.41	**0.82**	-0.85	-0.83	-0.73	-0.30	-0.80	-0.79	1		
FUM	-0.74	-0.59	-0.53	0.11	**0.88**	-0.96	-0.90	-0.93	-0.60	-0.76	-0.93	**0.84**	1.00	
FUG	-0.70	-0.57	-0.53	0.15	**0.87**	-0.98	-0.89	-0.94	-0.61	-0.74	-0.94	**0.84**	1.00	1

P <0.05

6.*CONCLUSION*

According to the obtained results, perennial ryegrass is a forage crop suitable for the production of ecologically clean biomass with a high forage value in the conditions of the Central Balkan Mountain. Through agrotechnological approaches, which are the subject of the presented research work, namely variety selection, foliar feeding and intercropping with legume grasses, the productive potential and quality parameters of perennial ryegrass forage can be significantly influenced. The genotypic factor is essential for the appearance of the culture in independent hay growing, and the results confirm the value of the local, Bulgarian selection and, accordingly, the adaptive potential in the improvement work with the culture.

As a result of the comparative testing of Bulgarian and foreign perennial ryegrass vcultivars, it was established that the Bulgarian tetraploid cultivars Tetramis and Tetrani exhibit the highest resistance and productivity in the conditions of the Central Balkan Mountain. The fodder obtained from them has a higher crude protein content.

Another main focus of the dissertation work is the influence of foliar feeding on the forage productivity of perennial ryegrass under independent cultivation. According to the results, foliar feeding significantly affected yield, basic chemical composition, fibrous structural components of cell walls and *in vitro* digestibility of dry matter. It has been established that bio-fertilization with Panamin Agro significantly increases the yield of fresh mass, as well as the concentration of crude protein in dry matter. Mineral foliar feeding with Nano Sulfur positively affects the participation of perennial ryegrass in the volume of the formed grass mass.

The results of the mixed cultivation of perennial ryegrass with legume grasses adapted to the conditions of the Central Balkan Mountain, in terms of the energy nutritional value of the grass stand, emphasize its high forage value and potential production importance for the mountainous regions of Bulgaria.

7. *CONCLUSIONS*

The data from the conducted scientific experiments lead to the following conclusions:

1. According to the conducted comparative cultivar test in the conditions of the Central Balkan Mountain, the genotypic factor, respectively the choice of cultivar, had a significant impact on the productivity and fodder quality in independent cultivation of perennial ryegrass. The cultivars Tetramis (69.28 cm) and Nui (67.93 cm) formed grass stands with the highest plant height values but the lowest phenotypic variability among years.
2. The plants of the Bulgarian tetraploid Tetramis and Tetrani cultivars have the highest participation in the obtained fodder mass and, accordingly, show the highest resistance and adaptability in mountain conditions.
3. The Bulgarian cultivars, such as the diploid standard c Harmoniya and the tetraploid Tetramis and Tetrani had equal productivity for a medium-long period of use. The grass stands of Nira and Bokser cultivars were proven to have a lower yield of fresh and dry matter compared to the standard (Harmoniya).
4. The fodder obtained from cultivar Belida has a higher crude protein content. The excess in the values of the indicator is 14.88% compared to the control. The cultivars Tetramis and Tetrani were respectively 1.86% and 1.58% higher in crude protein than the diploid standard.
5. In the conditions of the Central Balkan Mountain, the biomass of the cultivar Belida has the highest *in vitro* digestibility of dry matter, the lowest content of ADF and cellulose, which suggests the formation of fodder with high nutritional value.
6. It was found that bio-fertilization with Panamin Agro (250 g/da) significantly increased the yield of fresh mass and the height of the plants in perennial ryegrass, respectively by 16.87% and by 5.02%.

7. The mineral treatment with Nano Sulfur a rate of (100 ml/da) ensured an 88.18% presence of *Lolium perenne* L. in the composition of the grass mass, and the biofertilization with Panamin Agro (150 g/da) – 86.18%.

8. Treatment with Panamin Agro increases by up to 31.90% the crude protein content in the dry matter of perennial ryegrass and lowers the crude fiber content by up to 5.67%.

9. In the conditions of the Central Balkan Mountain, no significant difference was found in the productivity of perennial ryegrass grown as a monoculture or in double mixtures with bird's-foot-trefoil, white and red clover.

10. For the studied legume fodder crops, the yield of dry mass in the two-component mixtures with perennial ryegrass was proven to be higher compared to their independent grass stands. The excess in the values of the indicator are respectively 12.22% (in the mixture *Lolium perenne* L. + *Lolium corniculatus* L.), 14.02% (in the mixture *Lolium perenne* L. + *Trifolium repens* L.) and 22.23% (in the mixture *Lolium perenn*e L. + *Trifolium pratense* L.).

11. Red clover is a legume component (species) with the highest and most persistent presence in the grass stand in mixed cultivation with perennial ryegrass.

12. In monoculture cultivation, the relative share of *Lolium perenne* L. in the grass stand is 67.92%, and the share of weeds is 32.08%. In the two-component mixtures with bird's-foot-trefoil, white and red clover, the degree of weed infestation is from 13.00-28.24%.

13. Averaged over the period, white clover in monoculture grass stands had the highest crude protein content in the dry matter.

14. In the two-component mixtures, the biomass of perennial ryegrass with red clover had the highest protein content (151.50 g kg^{-1} DM). The excess in the values of the indicator was by 16.79% (compared to the mixture with white clover) and 4.65% (compared to the mixture with bird's-foot-trefoil).

15. The species composition of the mixture and the conditions in the growing area have been proven to influence the fiber composition of the cell walls, which is a determining factor for the digestibility of the fodder mass. The biomass of the mixture *Lolium perenne* L. + *Trifolium repens* L. had the highest dry matter digestibility.

16. In the monoculture grass stands of legume crops, white clover has the highest digestibility of dry matter. The excess in the values of the indicator is respectively by 8.40% compared to the red clover and by 5.81% compared to the bird's-foot-trefoil.

17. Over the study period, monoculture grass stands of red clover had the highest content of neutral-detergent fiber, and the biomass of bird's-foot-trefoil had the highest content of acid-detergent fiber, acid-detergent lignin and cellulose. The values of the indicators for white clover are the lowest compared to the other legume crops.

18. Monoculture grass stands with bird's-foot-trefoil had the highest gross energy content. With the lowest value of the indicator is the biomass of white clover, but with the highest content of exchangeable energy, FUM and FUG.

19. The fodder mass of perennial ryegrass in monoculture grass stands had a lower energy nutritional value compared to its mixtures with legume fodder crops. The most caloric were the grass stands of *Lolium perenne* L. with *Lotus corniculatus* L. Biomass also had the highest energy content, useful for the normal course of physiological needs in animals, as well as in the number of feed units for milk and growth in dry matter.

20. Correlation and regression relationships were established between the main qualitative and quantitative indicators in the forage from monoculture and mixed grass stands, namely: the amount of the fiber fraction correlates strongly negatively with the *in vitro* digestibility of dry matter, the energy value of the forage and the concentration of crude

protein. Crude protein content showed a statically significant influence on digestibility and the number of forage units in the forage mass.

8. *PRACTICE RECOMMENDATIONS*

As a result of the conclusions drawn, the following recommendations for practice can be indicated:

1. The Bulgarian tetraploid cultivars of perennial ryegrass Tetramis and Tetrani are suitable for growing in the conditions of the Central Balkan Mountain on pseudopodzolic soils.
2. The Panamin Agro bioproduct (250 g/da) can be recommended for inclusion in the technology of growing grain fodder crops in order to increase the productivity and quality of the fodder.
3. In the conditions of the Central Balkan Mountain, the mixture of perennial ryegrass with red clover has been proven to have the highest dry mass productivity.
4. The two-component mixture of perennial ryegrass and white clover has the highest nutritional value and digestibility of dry matter.
5. The developed graphical regression models for indicative determination of the composition and nutritional value of forage are suitable for practical purposes, because they can be used to predict some quality parameters through the botanical composition and yields of grass mass from single and mixed grass stands.

9. *CONTRIBUTIONS*

9.1. *Scientific and theoretical contributions of an original nature.*

1. Development was tracked, forage productivity was determined, and the quality and nutritional value of dry biomass of perennial ryegrass varieties under mountain conditions was evaluated.
2. The impact of mineral fertilizing with Nano Sulfur and foliar treatment with biofertilizer Panamin Agro on the growth, development, productivity and quality of perennial ryegrass on light gray pseudozolic soils was monitored.
3. Numerical parameters for growth rate, botanical composition and productivity of perennial ryegrass and leguminous forage grasses in single and mixed grass stands were established.
4. The main chemical composition was analyzed and the structural fibrous components of the cell walls in the biomass of perennial ryegrass after applied mineral and organic fertilizing were determined. The significance of the differences in the values of the investigated indicators by years and variants was also established.
5. The nutritional value and quality of perennial ryegrass in single and mixed grass stands with bird's-foot-trefoil, white and red clover were evaluated.
6. Correlation and regression relationships were established between main indicators of productivity and forage quality from grass stands of individual grain and legume fodder crops and their mixtures.

9.2. *Contributions of an applied nature.*

1. In the foothills of Bulgaria, tetraploid varieties of perennial ryegrass Tetramis and Tetrani have the best adaptability and resistance.
2. The positive effect of the action of the bioproduct Panamin Agro (250 g/da) in the perennial ryegrass is an alternative means of improving the productivity and quality of the biomass.

3. In the conditions of the Central Balkan Mountain, mixtures of perennial ryegrass with white and red clover are suitable for joint cultivation and obtaining a highly productive and high-quality fodder mass.

10. SCIENTIFIC PUBLICATIONS ON THE SUBJECT OF THE DISSERTATION:

Petkova M., 2022. Productivity of Perennial Ryegrass (*Lolium perenne* L.) Cultivars under Mountain Conditions. *Journal of Mountain Agriculture on the Balkans*, 25 (6) 258-269. https://jmabonline.com/en/article/wlnRXuulijFZBmvYqC77

Petkova M., T. Bozhanska, B. Bozhanski, M. Iliev, 2022. Chemical Composition and Energy Nutritional Value of Forage Crops under Mountain Conditions. *Journal of Mountain Agriculture on the Balkans*, 25 (5) 254-271. https://jmabonline.com/en/article/xTYwrVoyMxpS7hC2GICG

ABSTRACT

The research work aims to establish the possibilities for the production of ecologically clean forage biomass with high nutritional value in the conditions of the Central Balkan Mountain, through independent and mixed cultivation of perennial ryegrass.

To achieve the goal, this research was conducted to establish the suitability of Bulgarian and introduced cultivars of perennial ryegrass for the production of quality fodder; the impact of foliar feeding with fertilizers on the nutritional regime, productive potential and quality of perennial ryegrass forage mass; the opportunities of mixed cultivation of perennial ryegrass with adapted for mountain areas legumes forage grass and obtaining fodder with a high protein content for the needs of animal husbandry.

According to the obtained results, perennial ryegrass is a forage crop suitable for the production of ecologically clean biomass with a high forage value in the conditions of the Central Balkan Mountain. Through agro technological approaches, which are the subject of the presented research work, namely variety selection, foliar feeding and intercropping with legume grasses, the productive potential and quality parameters of perennial ryegrass forage can be significantly influenced. The genotypic factor is essential for the appearance of the culture in independent hay growing, and the results confirm the value of the local, Bulgarian selection and, accordingly, the adaptive potential in the improvement work with the culture.

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Another main focus of the dissertation work is the influence of foliar feeding on the forage productivity of perennial ryegrass under independent cultivation. According to the results, foliar feeding significantly affected the yield, basic chemical composition, fibrous structural components of cell walls and in vitro digestibility of dry matter. It has been established that bio-fertilization with Panamin Agro significantly increases the yield of fresh mass, as well as the concentration of crude protein in dry matter. Mineral foliar feeding with Nano Sulfur positively affects the participation of perennial ryegrass in the volume of the formed grass mass.

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