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BOTANICAL AND BASIC CHEMICAL COMPOSITION OF FORAGE FROM PERENNIAL GRASS CROPS GROWN IN MONOCULTURE AND MIXED GRASSLAND UNDER MOUNTAIN CONDITIONS

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Abstract

The experiment was carried out in order to evaluate the botanical composition and quality of fodder from perennial forage grasses (Festuca rubra L., Lolium perenne L., Dactylis glomerata L., Phleum pretense L.) grown in monoculture and in two-component mixtures with Trifolium pratense L. (50:50%) under mountain conditions. It was established that the studied grass species showed good adaptability and resistance to the specific soil and climate conditions of the experimental area. The relative share of grass species in monoculture meadows varied between 92.3% and 98.8%. The highest CP content in dry matter was recorded in Dactylis glomerata L. (101.4 g kg^{-1}) and Phleum pratense L. (97.9 g kg^{-1}). CP values exceeded the average by 6.1% and 2.4%, respectively. For the conditions of the Central Balkan Mountains, the mixtures of Festuca rubra L. and Phleum pretense L. with Trifolium pratense L. had the most balanced botanical composition (the ratio of grasses and legumes in the forage mass was 39.7:44.8% and 37.8:41.2%) and with the highest CP content. The indicator exceeded the average value by 37.8%, respectively 36.8%.

Key words*: perennial fodder grasses; botanical composition; basic chemical composition.*

INTRODUCTION

The livestock industry faces challenges as the demand for forage for livestock (Neto et al., 2021; Hisham et al., 2022). Perennial meadow grasses of the family *Poaceae* and *Fabaceae* are a cheap resource for meeting the food needs of farm animals. The choosing of suitable grasses and legumes is a significant factor in the high nutritional value of the forage (Pavlov, 1996). The microclimate and the specific soil characteristic in the growing area are factors determining the choice of grass and legume forage species when growing in monoculture and mixed grasslands.

To establish meadows in the conditions of the Central Balkan Mountains (Bulgaria), in monoculture or in mixtures, the perennial leguminous species, such as *Lotus corniculatus* L. and *Trifolium pratense* L. and the perennial grass species - *Festuca rubra* L. and *Phleum pratense* L. are some of the most suitable (Bozhanska & Churkova, 2019).

The biological croping of garsses as monocultures is the main cause for a vigorous weed infestation of the crops and limited nitrogen supply for plants (Petkova et al., 2023). The inclusion of legumes in mixed grasslands increases the dry matter yield, the non-structural carbohydrate concentration, improves the nitrogen supply of grasses (through symbiotic nitrogen fixation) and decreases the need to apply nitrogen fertilizers (Finn et al., 2013; Naydenova et al., 2013; Bélanger et al., 2014; Gungaabayar et al., 2023).

Perennial grass mixtures have higher productivity and resistance to invasive species compared to monocultures (Bélanger et al., 2014). The growth and development of plants in mixed crops are related to the morphological and physiological characteristics of the species, as well as to the more efficient use of natural resources (Naydenova et al., 2015; Churkova & Churkova, 2021).

An important advantage of mixed sown crops is their longevity, rich species composition, and quality of the grassland (Vasileva, 2014; Vasileva & Ilieva, 2016; Vasileva & Enchev, 2018). The concentration of nutrients and macronutrients depends on the ratio of the main plant components in plant biomass, the harvest stage and the method of cultivation (Tahir et

al., 2022; Olszewska, 2022; Olszewska & Mackiewicz-Walec, 2023).

Under the mountain conditions of Bulgaria, the two-component mixtures - *Lotus corniculatus* L. with *Festuca rubra* L. and *Trifolium pratense* L. with *Phleum pratense* L. have high adaptability, productivity, and an optimal ratio of plants in the grassland. The forage mass from the mixture of *Trifolium repens* L. with *Poa pratensis* L. has a high concentration of crude protein, minerals, and crude fat. The grassland has low values of fibrous structural elements in the composition of the plant cell and high *in vitro* digestibility of dry matter (Bozhanska, 2017b; Bozhanska et al., 2018).

The composition of the grass stand is essential to determine the effective uptake and assimilation of forage (Naydenova & Katova, 2013; Bozhanska, 2017b; Churkova & Churkova, 2022; Markov et al., 2024). Beyond these, it has been established that there is a direct dependence between the general condition of ruminant's body and the content of protein, calcium, and phosphorus in the dry matter of the forage (Slavkova & Shindarska, 2017; Slavkova et al., 2017). The nutritional value of plant biomass is a prerequisite for obtaining high quality meat and dairy products.

The purpose of the research was to analyze the botanical composition and the quality composition of some meadow grasses grown as monoculture and as two-component mixtures with *Trifolium pratense* L., in the foot-hill of the Central Balkan Mountain.

MATERIALS AND METHODS

The experiment was conducted in the period 2020-2023, in the experimental field of the Research Institute of Mountain Stockbreeding and Agriculture of Troyan (Bulgaria), on light gray, pseudopodzolic soils with pH = 4.2-5.5 (Penkov, 1988). The soils in the experimental area are light gray, pseudopodzolic. The content of the main nutrients in the soil layer was: from 0-20 cm - total N - 20.2 mg /1000 g, P_2O_5 - 2.4 mg/100 g, K_2O - 9.9 mg/100 g, humus - 1.44% and from 20-40 cm - total N - 8.6 mg/1000 g, P_2O_5 - 1.2 mg/100 g, K_2O - 5.9 mg/100 g, humus - 0.96% (Bozhanska, 2017a).

The objective of the study refers to four perennial species of grass forage (*Festuca rubra* L., *Lolium perenne* L., *Dactylis glomerata* L., *Phleum pratense* L.) grown as monoculture (100%) and in mixtures with red clover (*Trifolium pratense* L.) in a ratio of 50%:50%, under nonirrigated conditions.

The experimental variants included

Monoculture grass stands
1. Festuca rubra (*Festuca rubra* L.)
2. Perennial ryegrass (*Lolium perenne* L.)
3. Cock's foot (*Dactylis glomerata* L.)
4. Timothy (*Phleum pratense* L.)
Mixed grass stands
5. Festuca rubra (*Festuca rubra* L.) + Red clover (*Trifolium pratense* L.)
6. Perennial ryegrass (*Lolium perenne* L.) + Red clover (*Trifolium pratense* L.)
7. Timothy (*Phleum pratense* L.) + Red clover (*Trifolium pratense* L.)
8. Cock's foot (*Dactylis glomerata* L.) + Red clover (*Trifolium pratense* L.)

Sowing was done manually, by scattering. The sowing rates of the studied forage species were calculated based on 100% seed germination. Immediately after sowing, the sown areas were rolled for better contact of the seeds with the soil and to ensure simultaneous germination of the plants. The plot size was 5 m^2, laid out in 4 replications. Once a year (the last ten days of March) the monoculture crops were treated with 17 kg N/da and the mixed grasslands with a combined fertilizer of 12 kg N/da and 15 kg P_2O_5/da.

The grasslands were mowed at the beginning of the tasseling/ear formation period for grasses and the bud-formation period/blossoming period for legumes. The weed control during the vegetation was mechanical, intending to not allow additional chemical intervention on the plants.

The following indicators were studied

Botanical composition of the grass stand (%) – determined by plant weight, by analyzing grass samples by groups (grasses, legumes and weed vegetation), taken immediately before mowing.

The chemical composition of the dry mass according to the *Weende* method includes:

Crude protein (CP, g kg^{-1}) according to *Kjeldahl* (BDS/ ISO-5983); to decompose the organic matter, the sample is boiled with sulphuric acid in the presence of a catalyst. The acidic solution is alkalified with sodium hydroxide solution. The ammonia is distilled

and collected in a certain amount of H_2SO_4 (100 ml), the excess of which is titrated with a standard solution of sodium hydroxide. Alternatively, the separated ammonia is distilled in surplus of boric acid solution and then titrated with hydrochloric or sulphuric acid solution.

Crude fiber (CFr, g kg^{-1}) – the sample is treated sequentially with solutions of 1,25% (w/v) H_2SO_4 and 1,25% (w/v) NaOH. The residue is dried, ashed and weighed.

Crude fats (CF, g kg^{-1}) – were analyzed by extraction in a *Soxhlet* type extractor with a non-polar organic solvent. After the extraction, the sample is dried in a laboratory dryer at 95ºC to a constant weight.

Ash (g kg^{-1}) – decomposition of organic matter by gradual combustion of the sample in a muffle furnace at 550°C.

Moisture content (g kg^{-1}) – drying the sample in a laboratory dryer at a temperature of 105°C to a constant weight.

Dry matter (DM, g kg^{-1}) – empirically calculated from % of moisture.

Calcium (Ca, g kg^{-1}) – according to Stotz (complexometric).

Phosphorus (P, g kg^{-1}) – with a vanadate-molybdate reagent according to the method of Gerike and Kurmis (Sandev, 1979) – spectophotometer (*Agilent* 8453 UV – *visible Spectroscopy System*) measuring in the 425 ηm region.

Nitrogen-free extractable substances (NFE) = 100 – (CP, % + CFr, % + CF, % + Ash, % + Moisture, %), converted to g kg^{-1}.

Climate characteristics in the experimental area

The experimental territory belongs to the Pre-Balkan (mountain) climate region of the temperate-continental climate subregion (Sabev & Stanev, 1963). The average annual temperature is characterized by territorial differentiation (from north to south) with increasing altitude. The average of the annual temperatures is 10/11°C (Ninov, 1997). The distribution of precipitation is uneven with a maximum in summer (309 mm) and minimum (168 mm) in winter. Spring is relatively cool and well-supplied with rainfall.

The data in the experimental years indicate that the average annual temperatures exceed the multiannual ones (10.6ºC) by 0.4ºC to 2.0ºC (Table 1). The temperature values in March (3.2-7.8ºC) were suitable for germination and development of the studied forage species. The average temperature during the vegetation (March-October**)** was from 14.6ºC (2021) to 16.3ºC (2023) with an average for the experimental period of 15.3ºC and an average for a multiannual period of 14.8ºC. An increase of the temperature values, from 1.3ºC to 1.6ºC, was observed during July-October compared to those of a multiannual period.

Table 1. Average monthly air temperature (ºC) for the period 1990-2023

Years	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Average	Average for III – X
2020	0.4	4.4	7.1	9.4	14.7	17.8	20.4	21.1	17.8	12.7	5.2	3.8	11.2	***15.1***
2021	1.6	3.7	3.6	8.3	15.4	18.9	22.7	22.7	16.2	8.7	7.5	2.6	11.0	***14.6***
2022	0.8	3.8	3.2	10.3	15.9	19.8	22.1	21.8	16.3	12.0	8.3	4.4	11.6	***15.2***
2023	5.3	3.2	7.8	9.9	14.2	19.0	23.2	22.3	18.8	15.4	8.1	3.9	12.6	***16.3***
2020-2023	***2.0***	***3.8***	***5.4***	***9.5***	***15.1***	***18.9***	***22.1***	***22.0***	***17.3***	***12.2***	***7.3***	***3.7***	***11.6***	***15.3***
1990-2019	***-0.5***	***2.3***	***5.5***	***10.5***	***15.2***	***18.8***	***20.8***	***20.7***	***15.7***	***10.9***	***6.1***	***1.8***	***10.6***	***14.8***

The characteristic of weather during the experiment shows the variation of climate factors, which specifically affect the development, productivity, and quality of forage species.

The highest annual precipitation amount (712.9 mm) were reported in 2023 compared to the other experimental years, but also compared to the average annual norm for the period 1990-2019 (789.7 mm), the values are 76.8 mm lower (Table 2). The data indicate that the annual precipitation amount during the experimental period is lower by 33.3-167.6 mm compared to those for a multiannual period. Droughts in Bulgaria are observed in all seasons, which affects the physiological processes during the different pheno-phases of the individual development of forage species.

The lowest annual precipitation amount (545.3 mm) and vegetation precipitation amount (March-October - 379.9 mm) were registered in the third experimental year (2022) when the main components in the monoculture and mixed grasslands reached optimal development and increased their participation in the grassland.

Table 2. Monthly and annual precipitation amount (mm) for 1990-2023

Years	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Annual amount	Amount for III – X
2020	15.4	66.2	53.4	24.4	63.8	129	75.4	56.4	33.6	114.2	20.4	27.4	***679.6***	***550.2***
2021	82.8	25.6	47.7	57.0	82.8	64.8	12.4	56.2	11.8	72.8	23.6	68.6	***606.1***	***405.5***
2022	21.8	55.4	14.4	95.8	28.8	78.9	35.4	64.6	58.8	3.2	61.6	26.6	***545.3***	***379.9***
2023	12.4	27.8	25.8	82.6	174.5	132.4	27.6	50.2	28.4	1.8	81.2	68.2	***712.9***	***523.3***
2020-2023	***33.1***	***43.8***	***35.3***	***65.0***	***87.5***	***101.3***	***37.7***	***56.9***	***33.2***	***48.0***	***46.7***	***47.7***	***636.0***	***464.7***
1990-2019	***41.6***	***40.6***	***56.7***	***66.9***	***98.2***	***111.8***	***98.0***	***66.7***	***69.5***	***58.0***	***37.5***	***44.4***	***789.7***	***625.6***

For the study period, the annual and vegetation precipitation amounts were lower by 153.7 mm and 160.9 mm, respectively, compared to the values for a multiannual period (1990-2019).

Air temperature and precipitation are factors impacting the composition, density, and resistance of the studied plant species. In the experimental years, spring moisture offered optimal conditions for the formation of the first regrowth, both in monoculture and mixed grasslands.

Analysis of variance (ANOVA) was used for statistical processing of the data.

RESULTS AND DISCUSSION

Botanical composition of monoculture grasslands of perennial forage grasses

The botanical analysis data indicate that in the year of sowing (2020), the monoculture crops were heavily weeded (from 61.5% to 76.9%) and with a low share of the main grass crop (from 23.2% to 38.5%) in the grassland (Figures 1 and 2). *Phleum pratense* L. crops (the species with the highest productivity during the experimental period) had the lowest weed infestation degree, whereas *Dactylis glomerata* L. had the highest.

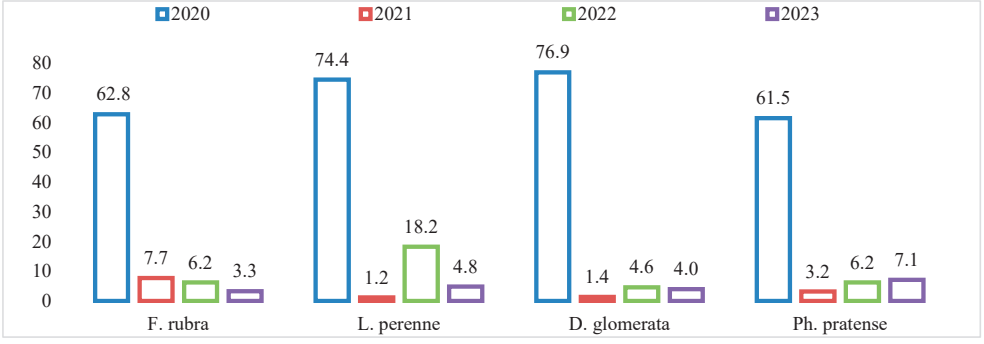

Figure 1. The relative share of weeds (%) in grasslands with monoculture grass species (by vegetation)

Under mountain conditions monoculture crops with *Festuca rubra* L. are distinguished by an increasing progression in the relative share of the main species in the grassland over the years (92.3% - 2021; 93.8% - 2022 and 96.7% - 2023). *Phleum pratense* L. is the only species with decreasing values of the indicator (96.8% - 2021; 93.8% - 2022 and 92.9% - 2023).

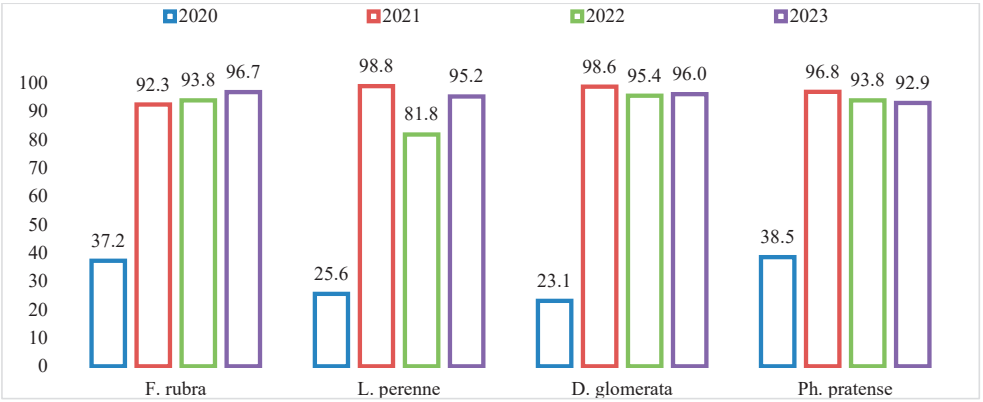

Figure 2. The relative share of grass species (%) in the monoculture grasslands (by vegetation)

So, the obtained results influence the assessment regarding the quality and nutritional value of the forage.

Given the global climate warming and the ojective to minimize the negative impact of the environment on forage productivity and quality, the identification of forage species with high adaptive potential in areas with variable and uneven precipitation distribution became a challenge (Huang et al., 2017; Ferner et al., 2018). The growth and the development of the plant as well as the forage production are highly dependent on moisture availability (Liu et al., 2023; Lumactud et al., 2023). The data from the present experiment indicate that the monoculture grasslands with *Lolium perenne* L. in the third vegetation (with 44.6% lower annual precipitation amount in the period of active vegetation compared to those in the long-term period) have the lowest share of the main species in the forage mass composition (81.8%). It can be considered that they are most susceptible to insufficient moisture availability supply compared to the monoculture grasslands with other tested grasses.

Dactylis glomerata L. plants were highly resistant and adaptable in mountain conditions, as well as tolerant to the acid reaction of the soil in the experimental area. A significant factor for this is the high and relatively stable share of the species in the forage mass (98.6% - 2021; 95.6% - 2022 and 96.0% - 2023) under conditions of both low and high moisture availability.

Botanical composition of mixed grasslands with perennial grasses and legumes

Perennial meadow grasses and legumes are valuable components in the composition of mixed grasslands, and the forage mass, that they form, is highly productive with a low degree of weed infestation (Albayrak et al., 2011; De Silva et al., 2023). The representatives of the *Fabaceae* family make a significant contribution to preserving soil fertility and enriching it with nitrogen, and grasslands enriched with legume forage crops are more sustainable, environmentally friendly, and with a higher energy value (Porqueddu et al., 2003; Andjelković et al., 2021; Marinova & Ivanova, 2023).

In the year of sowing, perennial grass species are characterized by a very slow rate of growth and development. The mixed grasslands had a high degree of weed infestation (51.6-86.6%), and the presence of the grass and legume components was from 9.3 to 24.7% and from 4.1 to 30.3%, respectively (Figures 3 and 4).

The two-component mixtures of *Festuca rubra* L. + *Trifolium pratense* L. and *Phleum pratense* L. + *Trifolium pratense* L. had a higher relative share of legumes than grasses. The ratio of species (grasses:legumes:weeds) in both grasslands is 21.0:27.4:51.6% and 13.6:30.3:56.1%, respectively.

The mixture of *Dactylis glomerata* L. and *Trifolium pratense* L. had the highest weed infestation degree in the first experimental year with from 20.6 to 35.0% compared to those of the other variants included in the study.

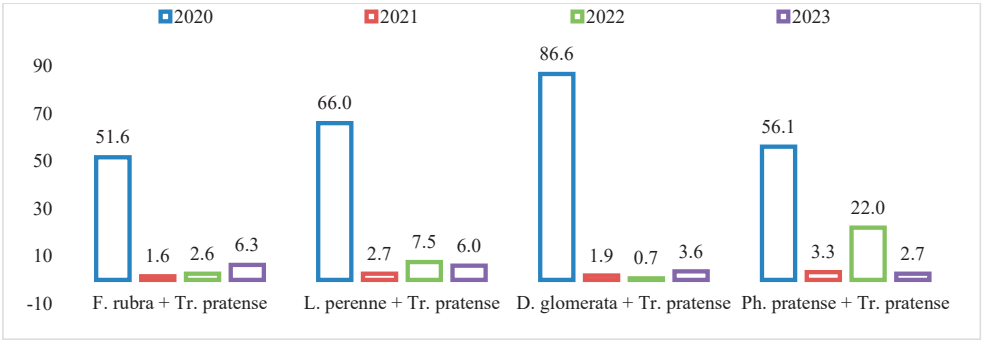

Figure 3. The relative share of weeds (%) in mixed grasslands (by vegetation)

The botanical composition of the grassland is an important factor related to the nutritional value and palatability of the produced forage mass, as well as the quality of the animal production (Rouquette, 2016; Bosire et al., 2019).

In the second experimental year, the relative share of legume species in the mixed grassland was from 26.0% to 60.9%. *Trifolium pratense* L. prevailed over the grass species only in the variants with *Festuca rubra* L. The highest presence of grasses in the mixed grasslands is *Lolium perenne* L. (71.2%) followed by *Dactylis glomerata* L. (64.8%). The grasslands with the mixture *Phleum pratense* L. + *Trifolium pratense* L. had the most balanced composition considering the grass-legume ratio (54.9:41.8%).

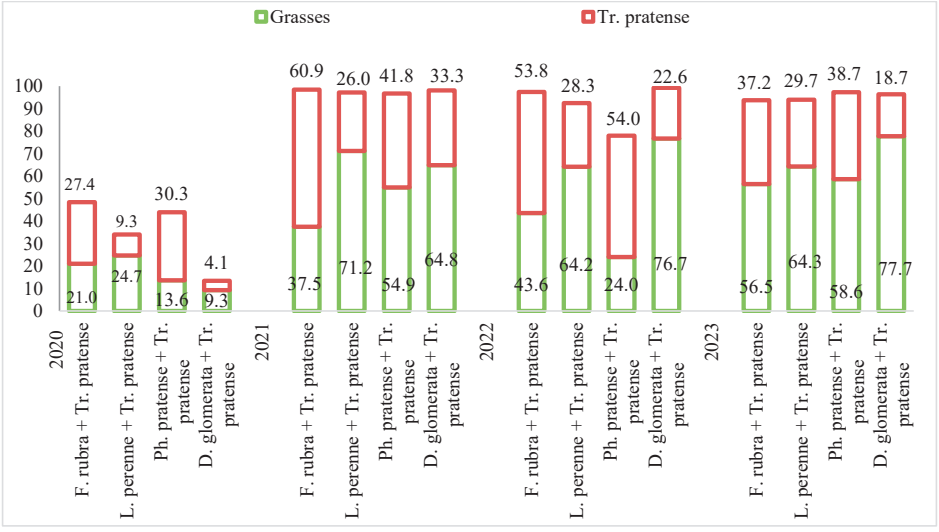

Figure 4. The relative share of grass and legume component (%) in mixed grasslands (by vegetation)

Legume forage crops are the main protein source in the composition of dry matter in mixed grasslands, and their share should be from 30 to 50% (Skamarokhova, 2018; Shamanin & Popova, 2021).

In this sense, in the third vegetation, the mixed grasslands of *Phleum pratense* L. + *Trifolium pratense* L. and *Festuca rubra* L. + *Trifolium pratense* L. are distinguished by a higher relative share of the legume species (< 50%) compared to the grass species. The grass-legume ratio in the two-component mixture of *Festuca rubra* L. with *Trifolium pratense* L. is 43.6:53.8% with a low degree of weed infestation (2.6%). The obtained results suggest the formation of a forage mass with a higher content of crude protein in the dry matter. In contrast, the *Phleum pratense* L. + *Trifolium*

pratense L. mixtures had the highest weed infestation level (22.0%), with lower values regarding the share of the grass species (24.0%) and almost identical values regarding the share of the legume species (54.0%) in the grassland.

In the perennial two-component mixtures of *Trifolium pratense* L. with *Lolium perenne* L. and *Dactylis glomerata* L., the grass species prevailed significantly in the forage mass compared to the legume. The ratio (grasses:legumes) in the indicator values is 64.2:28.3% and 76.7:22.6%, respectively.

In the third vegetation, the share of weeds in the forage mass of *Dactylis glomerata* L. + *Trifolium pratense* L. had the lowest values (0.7%).

In the fourth experimental year, the perennial grass mixtures of *Phleum pratense* L. + *Trifolium pratense* L. and *Festuca rubra* L. + *Trifolium pratense* L. - had the most balanced composition of the grass mass. The analysis data show almost identical values in the relative share of grasses and legumes in the two types of grasslands, respectively 58.6:38.7% and 56.5:37.2%. In the fourth vegetation, the grass species predominated in the share of the plant mass.

This trend is also preserved in the mixed grasslands of *Lolium perenne* L. + *Trifolium pratense* L. and *Dactylis glomerata* L. + *Trifolium pratense* L. The values regarding the ratio of the main components (grasses:legumes) in the two types of grasslands are 64.3:29.7% and 77.7:18.7%, respectively. During the year 2023, the mixed grasslands with *Festuca rubra* L. (6.3%) had the highest level of weed infestation. The lowest level of weed vegetation was registered in the variants *Phleum pratense* L. + *Trifolium pratense* L. (2.7%).

Main chemical composition of perennial forage species in monocultural and mixed grassland

The chemical composition of forage gives a real idea of its nutritional value (Table 3). The most significant in this regard is the content of crude protein in the composition of dry matter. The data from the chemical analysis indicate that in the **monoculture crops**, the crude protein content varies from 90.6 g kg^{-1} DM (*Lolium perenne* L.) to 101.4 g kg^{-1} DM (*Dactylis glomerata* L.) (Table 3). The values of the indicator are lower than the average (128.6 g kg^{-1} DM) by 21.2 to 29.5%, respectively. According to Babić et al. (2017) the ripening phase of cock's foot occurs later, which favours the yield of crude protein and increases the quality of the dry matter in the grassland.

In **two-component grass mixtures**, the amount of crude protein in dry matter significantly exceeded the average value of the indicator by 13.8% to 37.8%. The share of the legume component in the composition of the grass mass significantly increases the concentration of the protein fraction. Compared to monoculture, the values of this quality indicator in mixed grasslands were increased by:

- 91.6% (in the mixture of *Festuca rubra* L. + *Trifolium pratense* L.);
- 61.4% (in the mixture *Lolium perenne* L. + *Trifolium pratense* L.);
- 45.2% (in the mixture *Dactylis glomerata* L. + *Trifolium pratense* L.);
- 79.7% (in the mixture *Phleum pratense* L. + *Trifolium pratense* L.).

Table 3. Main chemical composition (g kg^{-1} DM) of perennial forage species in monoculture and mixed grassland average for the period 2020-2023

Variants	DM	CP	CF	CFr	Ash	NFE	Ca	P
Festuca rubra L.	906.5	92.5	29.0	368.6	59.8	356.7	13.3	4.7
Lolium perenne L.	907.4	90.6	25.6	413.3	45.3	332.6	7.9	2.4
Dactylis glomerata L.	909.0	101.4	30.2	387.0	47.2	343.2	16.1	1.8
Phleum pratense L.	908.8	97.9	22.0	405.6	40.1	343.2	9.9	1.9
F. rubra L.+*Tr. pratense* L.	903.9	177.2	28.7	368.6	69.1	260.2	20.0	2.7
L. perenne L.+*Tr. pretense* L.	906.2	146.3	30.1	359.5	54.4	315.8	17.6	1.7
Ph. pretense L.+*Tr. pretense* L.	902.0	175.9	26.0	301.4	57.6	341.0	18.7	2.3
D. glomerata L.+*Tr. pratense* L.	907.2	147.2	28.5	366.8	57.9	306.8	16.0	1.9
Mean	***906.4***	***128.6***	***27.5***	***371.4***	***53.9***	***324.9***	***14.9***	***2.4***
*$LSD_{0.05}$**	*0.6*	*2.1*	*1.2*	*5.3*	*1.8*	*5.2*	*0.9*	*0.9*
Significance of the differences in the values of the indicator over the years	*P >0.05*	*P< 0.001*	*P >0.05*	*P< 0.01*	*P >0.05*	*P< 0.05*	*P >0.05*	*P >0.05*
Significance of differences in indicator values among variants	*P< 0.01*	*P< 0.05*	*P >0.05*	*P< 0.001*	*P< 0.001*	*P< 0.01*	*P< 0.05*	*P< 0.05*

*$LSD_{0.05}$: Least Significant Difference at 5%

In the monoculture crops of perennial grasses (except for *Festuca rubra* L.), the crude fiber content exceeded the average value of the indicator by 4.2-11.3%. In the two-component mixtures, the values were from 301.4 g kg^{-1} to 368.6 g kg^{-1} and were by 0.8-18.8% lower compared to the average value of the indicator (371.4 g kg^{-1} DM). The obtained results are related to the type of grass component and the relative share of the legume species in the grassland.

Festuca rubra L. plants show adaptability and tolerance to acidic soils (the soils in the experimental area are pseudopodzolic with pH = 4.3), as well as the capacity for long-term use in mountain conditions (Katova & Vulchinkov, 2021). *Festuca rubra* L. showed the highest content of nitrogen-free extractable substances (356.7 g kg^{-1} DM), ash (59.8 g kg^{-1} DM) and phosphorus (4.7 g kg^{-1} DM) in monoculture cultivation. In contrast, the mixtures of *Festuca rubra* L. with *Trifolium pratense* L. had lower carbohydrate content in dry matter from 17.9 to 31.1% compared to the other mixed grasslands.

The content of calcium and phosphorus affects the biological value of the forage mass (Wyłupek et al., 2014). Our research shows that, the mixture *Festuca rubra* L. + *Trifolium pratense* L. had the highest average content of ash (69.1 g kg^{-1} DM), calcium (20.0 g kg^{-1} DM), and phosphorus (2.7 g kg^{-1} DM).

Nitrogen-free extractable substances are elements in the chemical composition that have an impact on the taste qualities of grass forage. It has been established that, the values of the indicator exceeded the average by 2.4-9.8% in the dry matter of monoculture crops. As shown in Table 3, in the mixed grasslands, only the mixture of *Phleum pratense* L. with *Trifolium pratense* L. had a higher nitrogen-free extractable substances content (by 4.9%) compared to the average value of the indicator (324.9 g kg^{-1} DM).

In the monoculture grasslands, the biomass of *Dactylis glomerata* L. had the highest calcium content (16.1 g kg^{-1} DM). The values of the indicator exceeded the average (14.9) by 8.1%. The obtained data confirm the findings of Bozhanska et al., (2022), namely that the grass stand of *Dactylis glomerata* L. had the highest content of Ca (18.3 g kg^{-1} DM) and P (1.6 g kg^{-1} DM) compared to some other cereal meadow grasses grown as monocultures. In the variants with two-component mixtures, Ca content was from 7.4 to 34.2% higher compared to the average value (14.9 g kg^{-1} DM).

Compared to monoculture crops, mixed grasslands also had a higher ash content. The values exceeded the average (53.9 g kg^{-1} DM) by 0.9-28.2%.

The difference in the results of the analyzed and empirically determined biochemical indicators in the monoculture crops and two-component mixtures is due to the share and age of the main grass species.

Average over the period, the sources of variation (years and variants) had a significant effect on the amount of CP ($P < 0.001$ and $P < 0.05$), CFr ($P < 0.01$ and $P < 0.001$) and NFE ($P < 0.05$ and $P < 0.01$) in the dry matter of the studied grasslands. The component composition of the grass mass has been proven to influence the concentration of DM ($P < 0.01$), Ash ($P < 0.001$), Ca and P ($P < 0.05$).

CONCLUSIONS

Perennial forage grasses grown in monoculture crops, in mountain conditions, are characterized by good adaptability and sustainability. The relative share of the main species in the grassland was from 92.3% to 98.8%. The highest crude protein content in the dry matter of monocultures was registered in *Dactylis glomerata* L. (101.4 g kg^{-1} DM) and *Phleum pratense* L. (97.9 g kg^{-1} DM).

In the conditions of the Central Balkan Mountain, the grasslands of the two-component mixtures *Festuca rubra* L. + *Trifolium pratense* L. and *Phleum pratense* L. + *Trifolium pratense* L. have the most balanced composition of the grass mass. For the study period, the grass-legume ratio was 39.7:44.8% and 37.8:41.2%, respectively. The biomass in variants with *Trifolium pratense* L. had higher crude protein content, suggesting the production of forage with higher nutritional value. The excess is from 13.8 to 37.8% compared to the average value of the sign (128.6 g kg^{-1} DM).

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