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QUALITATIVE CHARACTERISTICS OF FODDER FROM LEGUME AND GRASS CROPS IN PURE AND MIXED GRASS STANDS

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Abstract

The data from the analysis indicate that T. pratense in a pure crop shows the highest resistance and adaptability in foot-hill conditions, and in mixed with L. perenne maintains the highest presence. Grass stands of T. repens had the highest CP content (210.9 g kg^{-1}). The excess was by 9.6% (compared to L. corniculatus), 16.6% (compared to T. pratense) and 32.1% (compared to the average value). It is the crop with the lowest content of CFr (a decrease of 27.2% compared to the average value). The highest protein content was found in the biomass of L. perenne with T. pratense (151.5 g kg^{-1}), followed by the mixture with L. corniculatus (144.8 g kg^{-1}) and T. repens (129.7 g kg^{-1}). Grass stands of L. corniculatus and its mixtures with L. perenne had the highest content of DM. T. repens (63.7 g kg^{-1}) and the mixture of L. perenne with T. pratense (63.4 g kg^{-1}) had the highest ash content.

Key words*: legume and grass fodder crops, two-component mixtures, fodder quality.*

INTRODUCTION

The increased interest in ecologically clean agriculture leads to the search for crops with high adaptation and adjustability to the agro-ecological characteristics of the area. Cultivation of fodder species typical for mountain conditions is a suitable alternative for obtaining high yields and quality plant matter that satisfies the fodder needs of animals.

The competitive strength of the monoculture depends on the relative share of the species involved in the above-ground mass. Species interactions (synergistic or antagonistic) affect use regime, biodiversity, yield and weed infestation in grass stands (Finn et al., 2013; Black et al., 2017). The biological cultivation of garsses as monocultures is a prerequisite for vigorous weed infestation of crops and limited nitrogen supply of plants. The legume components increase the amount of nitrogen in the grass mass and a decrease in weed infestation degree (Arlauskienė et al., 2021). In a number of agroecosystems, nitrogen is an element that influences the provision of high yields in the formation of fodder mass (Thilakarathna, 2016), whereas mixed crops have a lower weed infestation degree (8%) compared to monocultures (43%) (Finn et al., 2012). Perennial ryegrass grown in mixed crops with legumes contributes to higher yields (Vasileva, 2015).

For the foot-hill conditions of Central Northern Bulgaria, Bulgarian selection populations of perennial ryegrass were studied in mixtures with red clover (Naydenova et al., 2010). For the experimental period, the legume component strongly dominated the grass stand. The perennial ryegrass in the mixture contributes to increase the quality characteristics of the fodder, as well as to maintain a sustainable balance between the cereal and leguminous components. Red clover (*T. pratense*) is the main species for maintaining fodder resources in the ecosystems of the foot-hill and mountain regions of Bulgaria (Naydenova & Bozhanska, 2014), white clover (*T. repens*) plays an important role in obtaining high-quality fodder (Mihovski & Sabeva, 2011), and the fodder mass of bird's-foot-trefoil (*L. corniculatus*) has a high nutritional value and digestibility ranging from 65% to 75% (Churkova et al., 2016; Churkova & Churkova, 2020).

The objective of the present experiment is to make a qualitative characterization of fodder resulted from legume and grass meadow species, grown as monocultures or in mixtures, under mountain conditions by following the botanical and chemical composition of the grass vegetation.

MATERIALS AND METHODS

The experiment was conducted in the period 2020-2022 at the Research Institute of Mountain Stockbreeding and Agriculture, Troyan (Bulgaria), on a light gray pseudopodzolic soil under non-irrigated conditions. The experiment was based on the block method, in 4 replications with a plot size of 5 m^2. The preparation of the experimental areas included: plowing in autumn, and in spring - disking and milling. The seeds were spread by hand, and then the land was rolled. For sowing the monoculture and two-component mixed grass stands, the following cultivars were used, respectively: perennial ryegrass, red clover, white clover and bird's-foot-trefoil.

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.)

The grass stands with monoculture were mowed manually in the initial phase of ear formation of grasses and bud-formation period until the beginning of blossoming of the legumes. Crops with mixed cultivation of grass and legume species were harvested in the phase of ear formation of the grasses and bud-formation period of the legume meadow grasses.

The following indicators were studied:

Botanical composition of the grass stand (%) - determined by 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 fiber (CFr, g/kg^{-1}) according to the *Weende* analysis - 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 protein (CP, g/kg^{-1}) according to Kjeldahl (according to 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 sulphuric acid, 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 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) and 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.
- NFE (Nitrogen-free extractable substances) = 100 – (CP, % + CFr, % + CF, % + Ash, % + Moisture, %), converted to g kg^{-1}.

Statistical analysis of data included one-way analysis of variance (ANOVA) and multiple comparison of means by least statistically significant difference ($LSD_{0.05}$).

RESULTS AND DISCUSSIONS

Botanical composition of monoculture grass stands of grass and legume fodder crops

Weed vegetation was present at a high percentage in the grass stand with the monocultures of grass and legumes in the year of sowing (Figure 1). In the variant with perennial ryegrass as a pure crop, the ratio of

grasses compared to weed vegetation was 56.5:43.5%, clearly indicating significant weed infestation of the grass stand. Of the legume crops, white clover had the lowest presence in the crop, and its ratio with weed vegetation was 57.5:42.5%. The ratio of components in the biomass of bird's-foot-trefoil was 61.2:38.8%, respectively legume: weed.

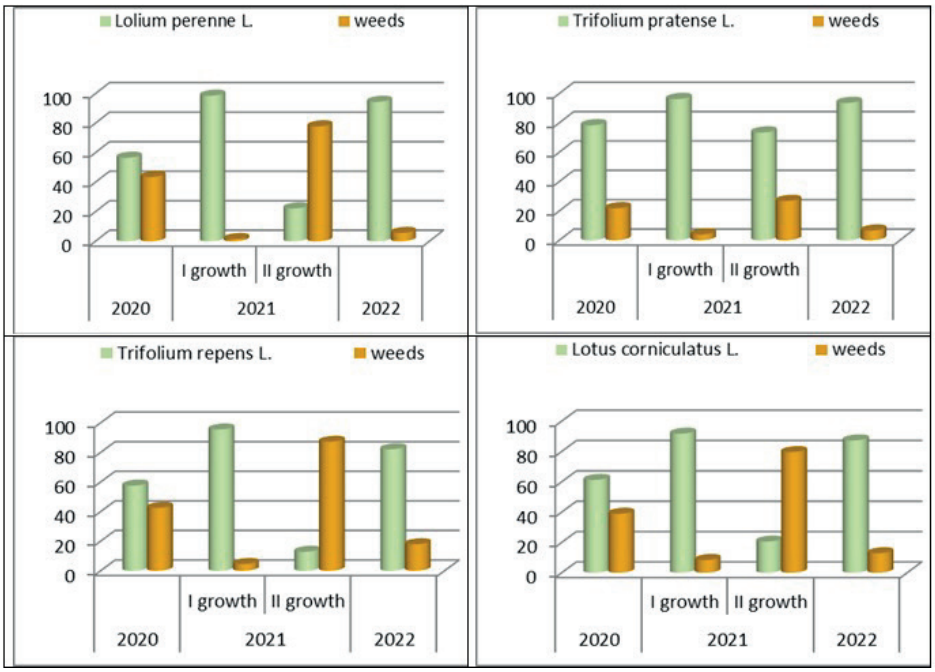

Figure 1. Botanical composition (%) of monoculture grass stands of grass and legume meadow species (by years)

Under favourable conditions and good seeding density, red clover significantly suppressed the development of weed vegetation (Naydenova, 2002). The results of the study indicate that in the first vegetation, the monoculture grass stands of red clover had a lower weed infestation degree, as the legume:weed ratio was 78.3:21.7%.

For the second experimental year, the weed infestation of the monoculture grass stands in the legume crops in the first cut ranged from 3.8% (*T. pratense*) to 8.3% (*L. corniculatus*). The results of the analysis indicate that the main crop had a high presence in the grass stand, namely: red clover with 96.2%, white clover with 95.5% and bird's-foot-trefoil with 91.7%.

L. perenne also showed a high share percentage (98.6%) in the formed biomass and a low percentage of weed infestation (1.4%).

In the second cut in 2021, the weed infestation rate was high in all tested variants of the legume and grass fodder crops. White clover had the largest share of weed vegetation in the grass stand (87.2%), whereas bird's-foot-trefoil and perennial ryegrass had close indicator values of 79.5% and 77.8%, respectively. Red clover had the highest share percentage in the plant biomass at a ratio of 73.3:26.7% between legumes: weeds, respectively.

In the third vegetation, the highest degree of weeding was registered in the monoculture grass stands with white clover (legume: weed ratio of 82.1:17.9%), followed by bird's-foot-trefoil (legume: weed ratio of 87.2:12.8%) and red clover (legume: weed ratio of 93.5:6.5%).

Perennial ryegrass had a high share (94.4%) in the above-ground mass compared to weed vegetation (5.6%).

Botanical composition of perennial two-component mixtures of grass and legume fodder crops

The proportional share and specific features of the ingredients in the composition of the grass

stands are decisive for the sustainability and use of the mixture (Bozhanska, 2017).

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 56.0:20.0%. *L.perenne* was also a dominant component (with 41.5%) in the mixture with white clover (23.3%), where was the highest percentage of weeds (35.2%) - Figure 2. Borawska-Jarmułowicz et al. (2012) tested meadow grasses in mixed crops indicating that perennial ryegrass (cv. Naki and Bajka) in the first experimental year appeared as an aggressive species compared to the other components in the mixture.

In the first experimental year, the mixed crop of perennial ryegrass with red clover had the most balanced ratio of both components (grasses: legumes - 43.1:36.8%). The grass stands also had the lowest weed infestation (20.1%) compared to the mixtures of perennial ryegrass with bird's-foot-trefoil (24.0%) and white clover (35.2%).

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 (47.6:47.6%) and the highest share of weed vegetation (4.8%) in the above-ground biomass. Perennial ryegrass dominated the spring regrowths of the white clover and bird's-foot-trefoil mixtures, as the share of grass, legume and weed vegetation was 80.9:17.0:2.1% and 85.7:13.4:0.9%, respectively. The variants of perennial ryegrass with bird's-foot-trefoil had the highest share of the grass component and the lowest weed infestation.

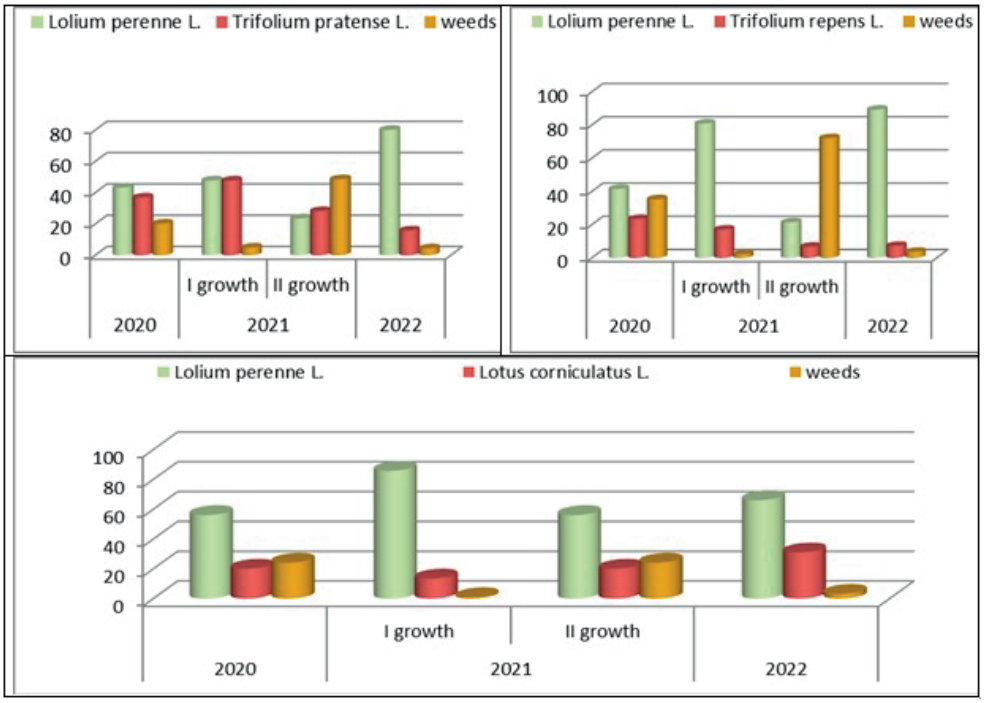

Figure 2. Botanical composition (%) of perennial two-component mixtures of grass and legume meadow species (by years)

In contrast, the second cut of the second vegetation marked significant weed infestation of the tested perennial grass-legume mixtures. The percentage of weeds varied from 24.0% (*L. perenne* + *L. corniculatus*) to 72.0% (*L. perenne* + *T. repens*). Perennial ryegrass had the highest share in the mixed crop with bird's-foot-trefoil, as the ratio of grass to legume component was 56.0:20.0%.

The mixture of perennial ryegrass with red clover also in this regrowth had the most balanced percentage ratio of the tested components with 23.4:28.1% compared to the other two variants. The lowest share of grass and legume crops in the grass stand was reported for the mizture of *L. perenne* + *T. repens*, respectively 21.3:6.7% at a high degree of weed infestation.

In the third experimental year, the weed infestation level in the mixed grass stands was relatively low. The values of the indicator vary from 3.1% (*L. perenne* . + *L. corniculatus*) to 4.3% (*L. perenne* + *T. pratense*). 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 89.2:7.2:3.6%, respectively.

Studies in mixed grass stands show that with the age of the grass stands, the share of grasses in the grassland increases and that of legumes and weeds decreases (Nešić et al., 2007). Compared with the second vegetation, 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 vegetation. The share of the perennial ryegrass was 80.0%, and the red clover was 15.7%.

In the third vegetation, the highest share of bird's-foot-trefoil was found in the grass stand (30.9%) with perennial ryegrass, compared to the first and second experimental years. The least weed infestation degree was registered in this mixture (3.1%), in comparison with other miztures, with a high share of perennial ryegrass (66.0%).

The botanical analysis of the monoculture grass stands with legume fodder crops indicated that *T. pratense* 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 32.0%) in mixtures with perennial ryegrass, followed by bird's-foot-trefoil (over 21.0%) and white clover (over 13.0%).

L. perenne as a monoculture, registered a high share of the grass component (over 67.0%) in the above-ground matter volume, with a share of weed vegetation less than 33.0%. In the mixed grass stands, perennial ryegrass maintained the highest share in the two-component mixture with bird's-foot-trefoil (<65%), followed by the mixture with white (<58%) and red clover (<48%).

Main chemical composition of monoculture and mixed grass stands of legume and grass fodder crops

The chemical composition of the fodder mass is a determining factor for the quality of the grass stands (Bozhanska, 2017).

In the year of sowing, in the monoculture grass stands of legume meadow grasses, white clover recorded the highest crude protein (214.1 g kg^{-1} DM), ash (83.6 g kg^{-1} DM), calcium (26,0 g kg^{-1} DM) and phosphorus (2.2 g kg^{-1} DM) - Table 1. The dry matter of the bird's-foot-trefoil contained the highest fiber content (338.9 g kg^{-1} DM) and the lowest level of macronutrients, such as calcium (14.7 g kg^{-1} DM) and phosphorus (0.7 g kg^{-1} DM).

Red clover as a monoculture has the lowest amount of protein fraction (181.5 g kg^{-1} DM) and the highest content of nitrogen-free extractable substances (391.6 g kg^{-1} DM), but compared to the other two-component grass stands, its mixture with perennial ryegrass had the highest values regarding the concentration of crude protein (139.8 g kg^{-1} DM) and mineral substances (83.7 g kg^{-1} DM).

Perennial ryegrass is grass fodder crops with great competitive ability (Bozhanska, 2017) and rapid development (Katova, 2016). During the first vegetation, it 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 (382.1 g kg^{-1} DM). The mixed grass stands of *L. perenne* with *L. corniculatus* had the lowest crude protein (127.0 g kg^{-1} DM), ash (71.5 g kg^{-1} DM) and NFE (350.5 g kg^{-1} DM) and those with white clover had the highest concentration of crude fat (35.2 g kg^{-1} DM) and calcium (21.5 g kg^{-1} DM).

In the second vegetation, in monoculture grass stands of *Trifolium* species, white clover again had the highest crude protein content (217.3 g kg^{-1} DM), followed by bird's-foot-trefoil (194.4 g kg^{-1} DM) and red clover (168.2 g kg^{-1} DM). Bird's-foot-trefoil biomass has the highest values of dry matter (926.0 g kg^{-1} DM) and crude fiber (413.3 g kg^{-1} DM), whereas red clover had the lowest with 1.6% respectively (for DM) and 36.5% (for CFr). The amount of macronutrients in the dry matter in the monoculture cultivation of legumes varied from 15.3 g kg^{-1} DM (*L. corniculatus*) to 17.8 g kg^{-1} DM (*T. pratense*) - for calcium and from 2.5 g kg^{-1} DM (*T. pratense*) to 3.2 g kg^{-1} DM (*T. repens*) - for phosphorus.

Animals are more willing to eat grass stands including many species from grass families of cereals and legumes (Baikalova et al., 2019).

Table 1. Chemical composition (g kg^{-1} DM) of legume and grass fodder crops in monoculture grass stands and perennial two-component mixtures, over the years and average for the period 2020-2022

Variants	DM	CP	CFr	CF	Ash	NFE	Ca	P
2020								
Lolium perenne L.	900.9	101.6	416.8	40.7	86.3	255.5	14.7	2.5
Trifolium pratense L.	894.1	181.5	220.6	24.9	75.5	391.6	17.0	1.0
Trifolium repens L.	890.7	214.1	197.8	29.7	83.6	365.5	26.0	2.2
Lotus corniculatus L.	899.4	196.2	338.9	30.5	60.8	273.0	14.7	0.7
L. perenne L.+*Tr. pratense* L.	894.0	139.8	257.1	31.3	83.7	382.1	12.5	2.0
L. perenne L.+*Tr. repens* L.	895.0	135.6	277.8	35.2	76.6	369.8	21.5	2.2
L. perenne L.+*L. corniculatus* L.	897.2	127.0	313.6	34.6	71.5	350.5	21.4	3.1
2021								
Lolium perenne L.	916.8	102.1	413.9	25.1	50.8	325.0	17.7	1.9
Trifolium pratense L.	911.1	168.2	302.8	44.9	69.8	325.6	17.8	2.5
Trifolium repens L.	916.6	217.3	349.8	26.4	70.4	252.8	16.6	3.2
Lotus corniculatus L.	926.0	194.4	413.3	36.0	59.2	223.2	15.3	2.9
L. perenne L.+*Tr. pratense* L.	920.7	147.4	486.2	22.3	59.9	204.9	12.2	2.2
L. perenne L.+*Tr. repens* L.	916.7	131.8	403.2	31.7	55.8	294.4	12.2	1.9
L. perenne L.+*L. corniculatus* L.	921.3	151.0	365.3	26.5	53.6	325.0	13.2	2.2
2022								
Lolium perenne L.	910.3	105.5	359.1	16.3	44.2	385.2	17.9	2.1
Trifolium pratense L.	907.1	192.9	381.4	19.3	39.0	274.5	21.2	3.4
Trifolium repens L.	911.1	201.4	259.8	26.5	37.2	386.2	21.1	2.6
Lotus corniculatus L.	911.9	186.9	278.4	23.6	45.2	377.8	10.1	3.3
L. perenne L.+*Tr. pratense* L.	913.3	167.3	388.3	11.3	46.7	299.7	14.5	1.9
L. perenne L.+*Tr. repens* L.	915.6	121.8	387.9	16.3	45.2	344.4	12.2	5.5
L. perenne L.+*L. corniculatus* L.	911.6	156.3	374.9	20.7	40.6	319.1	11.0	4.1
2020-2022								
Lolium perenne L.	909.3	103.1	396.6	27.4	60.4	321.9	16.8	2.1
Trifolium pratense L.	904.1	180.9	301.6	29.7	61.4	330.6	18.7	2.3
Trifolium repens L.	906.1	210.9	269.1	20.9	63.7	334.8	21.2	2.7
Lotus corniculatus L.	912.4	192.5	343.5	30.0	55.1	291.3	13.4	2.3
L. perenne L.+*Tr. pratense* L.	909.3	151.5	377.2	21.6	63.4	295.6	13.1	2.0
L. perenne L.+*Tr. repens* L.	909.1	129.7	356.3	27.7	59.2	336.2	15.3	3.2
L. perenne L.+*L. corniculatus* L.	910.0	144.8	351.3	27.3	55.2	331.5	15.2	3.1
Average	***908.6***	***159.0***	***342.2***	***26.4***	***59.8***	***320.3***	***16.2***	***2.5***
MAX	***912.4***	***210.9***	***396.6***	***30.0***	***63.7***	***336.2***	***21.2***	***3.2***
MIN	***904.1***	***103.1***	***269.1***	***20.9***	***55.1***	***291.3***	***13.1***	***2.0***
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 between 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

Multi-species cenoses are more productive and more stable than single-species ones provide a well-balanced nutritious food (Khramoj et al., 2019; Eichler-Löbermann et al., 2020). Plants from the Fabaceae family synthesize about 1.5-

3 times more protein than Poaceae (Stagnari et al., 2017; Kulkarni et al., 2018).

In the second vegetation, monoculture grass stands with perennial ryegrass were lower in ash (5.4 to 17.9%) and phosphorus (2.1 to 18.0%), but richer in nitrogen-free extractable substances (with from 0.4 to 58.6%) and calcium (with from 33.7 to 45.3%) compared to the mixed ones. Associated with legume fodder crops, the crude protein in the dry matter of its two-component mixtures increased from 29.1% (*L. perenne* + *T. repens*) to 48.0% (*L. perenne* + *L. corniculatus*) , and the crude fiber content varied from 365.3 g kg^{-1} DM (*L. perenne* + *L. corniculatus*) to 486.2 g kg^{-1} DM (*L. perenne* + *T. pratense*).

In the third vegetation, the maximum values of crude protein (201.4 g kg^{-1} DM), crude fat (26.5 g kg^{-1} DM) and nitrogen-free extractable substances (386.2 g kg^{-1} DM) were found in the monoculture grass stands with white clover. The biomass is distinguished by the lowest content of crude fiber (259.8 g kg^{-1} DM) and mineral substances (37.2 g kg^{-1} DM).

Grass stands with *T. pratense* had the highest fiber content (381.4 g kg^{-1} DM), calcium (21.2 g kg^{-1} DM) and phosphorus (3.4 g kg^{-1} DM) and low concentration of crude fat (19.3 g kg^{-1} DM) and nitrogen-free extractable substances (274.5 g kg^{-1} DM).

The grass mass of *L. corniculatus* had the lowest crude protein content (7.8% lower than the maximum indicator value) in dry matter and a relatively low crude fiber concentration (37.0% lower than the maximum value of the indicator) compared to that of the other legume grasses grown as monocultures.

In the third vegetation, the share of *L. perenne* in the white clover grass stand was < 89.0%, which affected the percentage of protein in the fodder mass. The mixture, compared to the others included in the experiment (*L. perenne* + *T. pratense*; *L. perenne* + *L. corniculatus*), had the highest content of dry matter (915.6 g kg^{-1} DM), NFE (344.4 g kg^{-1} DM) and phosphorus (5.5 g kg^{-1} DM), but poorest in crude protein (121.8 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 (167.3 g kg^{-1} DM) and calcium (14.5 g kg^{-1} DM) was the highest, and that of nitrogen-free extractable substances (299.7 g kg^{-1} DM) and phosphorus (1.9 g kg^{-1} DM) – the lowest. In the *L. perenne* + *T. pratense* mixture, the following regularity was observed: the high protein content was combined with the highest crude fiber content (388.3 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. In Norway, mixed grass stands dominated by perennial ryegrass include species (*Agrostis capillaris* L., *Trifolium pratense* L., *Trifolium repens* L.) that balance the composition of the grass stand, increase the productivity and quality of the fodder mass (Jørgensen et al., 2019).

The mixture *L. perenne* + *L. corniculatus* recorded the lowest content of fiber fraction (374.9 g kg^{-1} DM) and calcium (11.0 g kg^{-1} DM), and the highest of crude fat (20.7 g kg^{-1} DM).

The amount of mineral substances in the mixed grass stands varied from 40.6 g kg^{-1} DM (*L. perenne* + *L. corniculatus*) to 46.7 g kg^{-1} DM (*L. perenne* + *T. pratense*).

Averaged over the period, white clover in monoculture grass stands with legume fodder crops had the highest dry matter crude protein content (210.9 g kg^{-1}). The excess is respectively 9.6% (compared to bird's-foot-trefoil), 16.6% (compared to red clover) and 32.6% (compared to the average). The difference in the values of the indicator 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 (151.5 g kg^{-1} DM) followed by its mixture with bird's-foot-trefoil (144.8 g kg^{-1} DM) and white clover (129, 7 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 (269.1 g kg^{-1} DM) was found in the dry mass of white clover from the group of legumes. The decrease was 27.2% compared to the average value of the indicator. In the mixed grasslands, the amount of crude fiber was 2.6% higher (*L. perenne* +

L. corniculatus) to 10.2% (*L. perenne* + *T. pratense*) compared to the average (342.2 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 *L. corniculatus* (912.4 g kg^{-1} DM) and its mixtures with *L. perenne* (910.0 g kg^{-1} DM) had the highest dry matter content. The excess in the average values of the indicators were by 0.2-0.4% respectively. White clover (63.7 g kg^{-1} DM) and the mixture of perennial ryegrass with red clover (63.4 g kg^{-1} DM) recorded the highest ash content with an excess of 6.6 to 7.2% over the average of the indicator.

CONCLUSIONS

In monoculture cultivation, the relative share of *L. perenne* in the grass stand was over 67.0%, and in mixed crops it maintained the highest share in the two-component mixture with bird's-foot-trefoil (<65%), followed by the mixture with white (<58%) and red clover (<48%).

Monoculture grass stands of *T. pratense* show the highest resistance and adaptability in foot-hill conditions, and in mixed grass stands with *L. perenne* it retained the highest share (over 32.0%).

Averaged over the period, white clover in monoculture grass stands had the highest crude protein content (210.9 g kg^{-1}) in the dry matter. In the two-component mixtures, the biomass of perennial ryegrass with red clover had the highest protein content (151.5 g kg^{-1} DM). The excess in the values of the indicator was by 16.8% (compared to the mixture with white clover) and 4.6% (compared to the mixture with bird's-foot-trefoil).

1. When growing *L. perenne* in two-component mixtures with L. corniculatus, *T. repens* and *T. pratense*, the proportion of unwanted vegetation in the obtained biomass decreases significantly compared to that of its monoculture cultivation.

2. The mixed cultivation of *L. perenne* with the indicated medium-long-lasting leguminous grasses adapted to the area positively affects the main indicators of forage quality of the obtained biomass. The biomass of the mixture of *L. perenne* and *T. pratense* has the highest protein content. The studied mixtures are also distinguished by a more balanced protein and fiber content, which causes a higher digestibility of the feed.

3. *T. pratense* can be defined as the most suitable legume component for creating hay mixtures with *L. perenne* for the foothill conditions of the Central Balkan Mountain.

REFERENCES

Arlauskienė, A., Jablonskytė-Raščė, D., Šarūnaitė, L., Toleikienė, M., Masilionytė, L., Gecaitė, V., & Kadžiulienė, Ž. (2021). Perennial forage legume cultivation and their above-ground mass management methods for weed suppression in arable organic cropping systems. *Chemical and Biological Technologies in Agriculture*, 8, 34. https://doi.org/10.1186/s40538-021-00228-5.

Baikalova, L. P., Edimeichev, Yu. F., & Mashanov, A. I. (2019). Assessment of the yield of cultivated pastures in the conditions of Krasnoyarsk forest-steppe *Vestnik KrasGAU*, 8, 52-8.

Black, A. D., Anderson, S., & Dalgety, K.S. (2017). Identification of pasture mixtures that maximise dry matter yield. *Journal of New Zealand Grasslands*, 79, 103-109. https://researcharchive.lincoln.ac.nz/bitstream/handle/10182/9756/Black_IdentificationOfPasture_2017.pdf?sequence=3&isAllowed=y

Bozhanska, T. (2017). Productivity and quality characteristics of forage grasses and grass mixtures for the conditions of the Middle Balkan Mountain. Dissertation. Troyan.

Borawska-Jarmułowicz, B., Janicka, M., & Mastalerczuk, G. (2012). Yielding of grass cultivars in pure stands and phenologically different meadowmixtures in the first year of utilization. *Grassland Science in Europe*, 17, 88-90.

Churkova, B., Bozhanska, T., & Naydenova, Y. (2016). Feeding value of bird's–foot trefoil (*Lotus corniculatus* L.) cultivar under conditions of the central northern part of Bulgaria. *Banat's Journal of Biotechnology*, VII (14), 38-45. https://www.bjbabe.ro/wp-content/uploads/2016/10/38_CHURKOVA.pdf

Churkova, B., & Churkova, K. (2020). Energy Nutritional Value of Grass Mixtures with Different Ratio of Components. *Journal of Mountain Agriculture on the Balkans*, 23,3, 142-154.

Eichler-Löbermann, B., Busch, S., Jablonowski, N. D., Kavka, M., & Brandt, C. (2020). Mixed cropping as affected by phosphorus and water supply. *Agronomy*, 10, 10, 1506 doi:10.3390/agronomy10101506.

Finn, J.A., Kirwan, L., Connolly, J., Sebastià, T.M., Helgadóttir, Á., Lüscher, A., & participating sites in

COST 852. (2012). Four-species grass-clover mixtures demonstrate transgessive overyielding and weed suppression in a 3-year continental-scale experiment. *Grassland Science in Europe*, 17, 64-66.

Finn, J. A., Kirwan, L., Connolly, J., Sebastià, T. M., Helgadottir, A., Baadshaug, H. O., Bélanger, G., Black, D. A., Brophy, C., Collins, P. R., Čop, J., Dalmannsdóttir, S., Delgado, I., Elgersma, A., Fothergill, M., Frankow-Lindberg, E. B., Ghesquiere, A., Golinska, B., Golinski, P., Grieu, P., Gustavsson, M. A., Höglind, M., Huguenin-Elie, O., Jørgensen, M., Kadziuliene, Z., Kurki, P., Llurba, R., Lunnan, T., Porqueddu, C., Suter, M., Thumm, U. & Lüscher, A. (2013). Ecosystem function enhanced by combining four functional types of plant species in intensively managed grassland mixtures: a 3-year continentalscale field experiment. *Journal of Applied Ecology*, 50, 365-375.

Jørgensen, M., Leraand, K. M., Ergon, Å. & Bakken, K. A., (2019). Effects of including perennial ryegrass (*Lolium perenne* L.) in different species mixtures on yield, feed quality and botanical composition in first year of ley. *Grassland Science in Europe*, 2, 152-154. https://www.researchgate.net/profile/Pierre-Mariotte/publication/341986864_Improving_sown_grasslands_through_breeding_and_management/links/5f734835458515b7cf5819da/Improving-sown-grasslands-through-breeding-and-management.pdf#page=180

Katova, A. (2016). Study of growth and development of perennial ryegrass grown in pure stand and in mixtures with lucerne. *Journal of Mountain Agriculture on the Balkans,* 19 (2), 111-135.

Khramoj, V. K., Rakhimova, O. V., & Sikharulidze, T. D. (2019). Photosynthetic activity of two-and three-component vetch and grasses mixed crops in Central Non-chernozem zone. *AgrarianScience*, 4, 52-54.

Kulkarni, K. P., Tayade, R., Asekova, S., Song, J. T., Shannon, J. G., & Lee, J. D. (2018). Harnessing the potential of forage legumes, alfalfa, soybean, and cowpea for sustainable agriculture and global food security. Frontiers in *Plant Science*, 9, 1314 doi:10.3389/fpls.2018.01314.

Mihovski, Tsv., & Sabeva, M. (2011). New technological approaches in creating a mixed crop of white clover and perennial ryegrass. *Journal of Mountain Agriculture on the Balkans,* 14, 3, 541-547.

Naydenova, G., (2002). Study of the impact of some agrobiological factors in the cultivation of red clover (*Trifolium pratense* L). Dissertation, Troyan.

Naydenova, G., Mitev, D., & Katova, A. (2010). Testing selection populations of red clover and perennial ryegrass in mixtures. *Bulgarian Journal of Crop Sciences*, 47, 331-337.

Naydenova, G., & Bojanska, T., (2014). Selection for resistance to powdery mildew (*Erysiphe polygoni* dc.) in red clover (*Trifolium pratense* L.). *Agricultural Science*, 47, 1, 40-46.

Nešić, Z., Tomić, Z., Vučković, S., Krnjaja, V., Josipović, S. & Ružić-Muslić, D. (2007). Changes in botanical composition of alfalfa mixtures depending on the species and N fertilization. Biotechnology in Animal Husbandry, 23, 5-6, 365-374.

Sandev, S., (1979). Chemical methods of forage analysis. S., Zemizdat, 189.

Stagnari, F., Maggio, A., Galieni, A., & Pisante, M. (2017). Multiple benefits of legumes for agriculture sustainability: an overview. *Chemical and Biological Technologies in Agriculture*, 4, 1, 1-13 doi:10.1186/s40538-016-0085-1.

Thilakarathna, S. M., McElroy, S. M., Chapagain, T., Papadopoulos, A. Y. & Raizada, N. M. (2016). Belowground nitrogen transfer from legumes to non-legumes under managed herbaceous cropping systems. A review. *Agronomy for Sustainable Development*, 36, 4, 1-16.

Vasileva, V., 2015. Morphological parameters and rations in some mixtures with subclover, *Science international*, 3, 4, 107-112 DOI: 10.17311/sciintl.2015.107.112