

Quality and nutritive value analysis of perennial forage grasses under mountain conditions

Tatyana Bozhanska* and Biser Bozhanski

Agricultural Academy, Research Institute of Mountain Stockbreeding and Agriculture – Troyan, 5600 Troyan, Bulgaria

*Corresponding author: tbozhanska@mail.bg

Abstract

Bozhanska, T. & Bozhanski, B. (2026). Quality and nutritive value analysis of perennial forage grasses under mountain conditions. *Bulg. J. Agric. Sci.*, 32(2), 306–316

The aim of the present study is related to the quality assessment of forage biomass from sown hay grasslands. The content of fibre structural components of cell walls and energy nutritive value of four species of perennial meadow grasses (*Festuca rubra* L., *Lolium perenne* L., *Dactylis glomerata* L. and *Phleum pratense* L.), grown in pure stands under conditions of the Middle Balkan Mountains (Bulgaria) were investigated.

On average over the period, the lowest content of neutral-detergent fibre (NDF), acid-detergent fibre (ADF) and acid-detergent lignin (ADL) was the forage mass of *Lolium perenne* L. Significant differences were found in the amount of NDF and ADL in the biomass of the perennial forage species. The mean values of these parameters ranged from 554.8 g kg⁻¹ DM to 634.0 g kg⁻¹ DM and from 41.1 g kg⁻¹ DM to 64.1 g kg⁻¹ DM, respectively.

Festuca rubra L. grasslands are proven to have the highest hemicellulose content. The exceedance of the values of the indicator compared to the average (256.1 g kg⁻¹ DM) was 14.8%. For the experimental period, the biomass of *Dactylis glomerata* L. with the lowest cellulose content (269.7 g kg⁻¹ DM) and the highest in vitro dry matter digestibility (631.8 g kg⁻¹ DM). The biomass of *Phleum pratense* L. and *Dactylis glomerata* L. on with the highest energy nutritive value.

Fresh leaf mass of *Lolium perenne* L. was found to have the highest total plastid pigment content (280.21 mg/100 g FW). The excess over the mean value of the indicator was 22.1%.

Keywords: perennial meadow grasses; forage quality; digestibility; plastid pigments

Introduction

The changing conditions of the environment, as well as the pursuit of efficient and ecological forage production, require the selection of species with optimal productivity, quality and nutritional value (Akalın, 2014; Naydenova and Katova, 2015; Severoglu and Gullap, 2023).

Many species of *Poaceae* family have a wide range of distribution, optimal development and good seasonal productivity (Nazli et al., 2020; Robins et al., 2020; Churkova and Churkova, 2024a; Churkova and Churkova, 2024b). A large part of the plants are long-lived, drought tolerant

and with great ecological plasticity (Mitev and Naydenova, 2014; Khan et al., 2019). Dense thatch protects the soil from water and wind erosion and suppresses the development of weed vegetation (Kosolapov et al., 2021). Perennial types of meadow grasses possess a number of valuable qualities for the production of forage of good quality and favorable chemical composition. They are a cheap source of ecologically clean biomass that do not cause tympanitis and are readily accepted by animals. The carbohydrate content in the dry matter is high, which allows for independent silage and storage of the grass mass (Țîței, 2018; Katova, 2023a).

Sown grasslands of perennial meadow grasses form a forage mass with high productive potential and low cost (Scordia and Cosentino, 2019; Petkova and Iliev, 2025). As pure crops (on their own), they are grown less often and on limited areas (Kostov and Pavlov, 1999). Red fescue (*Festuca rubra* L.), perennial ryegrass (*Lolium perenne* L.), Cocksfoot (*Dactylis glomerata* L.) and timothy (*Phleum pratense* L.) are fodder crops with high adaptability in the mountainous regions of Bulgaria (Tenikecier and Ates, 2018; Bozhanska and Churkova, 2019). Plant density, height and foliage are factors that influence the quality, nutritional value and digestibility of forage (Katova, 2023b). Grown in separate crops, without fertilization (under mountain conditions), the relative share of these species varies from 92.3% to 98.8%, and the crude protein content – from 90.6 g kg⁻¹ DM (*Lolium perenne* L.) to 101.4 g kg⁻¹ DM (*Dactylis glomerata* L.) (Bozhanska et al., 2024).

Perennial meadow grasses are characterized by a high content of fibrous structural components in the dry matter, which mainly depends on the phenological stage of plant harvest (Katova and Naydenova, 2017a). The concentration of neutral-detergent fibers, acid-detergent fibers and lignin has been proven to vary during the vegetation period, correlates negatively with the digestibility of the forage mass and has a significant impact on the quantitative and qualitative parameters of the obtained animal production (Rusinovci et al., 2016; Billen et al., 2018; Rivero et al., 2019; Perotti et al., 2021).

The purpose of the study is to determine the content of the structural fiber components of the cell walls, the quality and the energy nutritional value of one of the country's main perennial meadow grasses, under the mountain conditions of Central Northern Bulgaria.

Material 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).

Agrochemical soil analysis

The soils in the area of experience are one of the poor in the country, making them unfavorable for agricultural crops because of their acidic (pH_{H₂O} = 5.2–5.5; pH_{KCL} = 4.3–4.4) reaction and insufficient water-air regime (Table 1). Penkov (1988) found them suitable for growing forage grasses because they provide high productivity.

The soils in the experimental area are light gray, pseudo-podzolic. The content of the main nutrients in the soil layer was: from 0–20 cm – total N – 20.2 mg /1000 g, P₂O₅ – 2.4

Table 1. Agrochemical soil analysis (0–40 cm)

Soil characteristics	pH		Σ N – NH ₄ + NO ₃ mg/kg ⁻¹	P ₂ O ₅ mg/100	K ₂ O mg/100	Humus %
	H ₂ O	KCL				
Soil layer						
0–20	5.3	4.4	20.2	2.4	9.9	1.44
20–40	5.2	4.3	8.6	1.2	5.9	0.96

Source: Authors' own elaboration

mg/100 g, K₂O – 9.9 mg/100 g, humus – 1.44% and from 20–40 cm – total N – 8.6 mg/1000 g, P₂O₅ – 1.2 mg/100 g, K₂O – 5.9 mg/100 g, humus – 0.96% (Bozhanska, 2017).

Climate characteristics in the experimental area

The experimental territory belongs to the Pre-Balkan (mountain) climate region of the temperate-continental climate subregion (Sabev and Stanev, 1963). The average annual temperature is characterized by territorial differentiation (from north to south) with increasing altitude. The average annual temperatures are 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 characterization of weather conditions during the years of the study shows variation in temperature and precipitation values, which specifically affect the development, productivity and quality of forage crops.

In the year of sowing, the average air temperature in March (7.1°C) allowed timely and optimal seed germination in the studied forage crops (Table 2). In the second and third experimental years, the onset of vegetation in grass species was associated with a lower value regarding the mean monthly temperature (3.7–3.8°C) and rainfall (25.6–55.4 l/m²) compared to the first and fourth years. In 2023, the average air temperature in March (the beginning of vegetative development of perennial grasses) was 0.7–4.6°C higher (compared to the previous years), and the recorded rainfall amount was low at 25.8 l/m². The year was characterized by the highest annual mean (12.6°C) and growing season air temperature (March–October = 16.3°C), as well as the highest annual rainfall (712.9 l/m²) compared to the other experimental years.

In the second experimental year (2021), the mean annual air temperature (11.0°C) was the lowest with a rainfall amount of 606.1 l/m². With the lowest annual (545.3 l/m²) and growing season precipitation amount (March–October = 379.9 l/m²) was the third experimental year (2022), when the major components in monoculture grasslands reached optimum development and increased their participation in the grassland growth.

The objective of the study refers to four perennial species

Table 2. Agro meteorological conditions for the period of study – temperature (°C) and rains (l/m²)

Months	2020		2021		2022		2023	
	Temperature	Rains	Temperature	Rains	Temperature	Rains	Temperature	Rains
January	0.4	15.4	1.6	82.8	0.8	21.8	5.3	12.4
February	4.4	66.2	3.7	25.6	3.8	55.4	3.2	27.8
March	7.1	53.4	3.6	47.7	3.2	14.4	7.8	25.8
April	9.4	24.4	8.3	57.0	10.3	95.8	9.9	82.6
May	14.7	63.8	15.4	82.8	15.9	28.8	14.2	174.5
June	17.8	129.0	18.9	64.8	19.8	78.9	19.0	132.4
July	20.4	75.4	22.7	12.4	22.1	35.4	23.2	27.6
August	21.1	56.4	22.7	56.2	21.8	64.6	22.3	50.2
September	17.8	33.6	16.2	11.8	16.3	58.8	18.8	28.4
October	12.7	114.2	8.7	72.8	12.0	3.2	15.4	1.8
November	5.2	20.4	7.5	23.6	8.3	61.6	8.1	81.2
December	3.8	27.4	2.6	68.6	4.4	26.6	3.9	68.2
Average/ Summ	11.2	679.6	11.0	606.1	11.6	545.3	12.6	712.9

Source: Authors' own elaboration

of grass forage – red fescue (*Festuca rubra* L.), perennial ryegrass (*Lolium perenne* L.), cocksfoot (*Dactylis glomerata* L.), timothy (*Phleum pratense* L.), grown as monoculture (100%) under nonirrigated conditions.

The experimental variants included:

1. *Festuca rubra* L. (FR)
2. *Lolium perenne* L. (LP)
3. *Dactylis glomerata* L. (DG)
4. *Phleum pratense* L. (PP)

Sowing was done manually, scattered (15–20 March). 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 m², laid out in 4 replications.

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

Given the soil characteristics in the experimental area, we implemented a spring mineral fertilization with 170 kg N/ha (NH₄NO₃) in the year of sowing.

Indicators of study

The fibrous structural elements in the plant cell are analyzed in laboratory: Neutral Detergent Fibers (NDF, g kg⁻¹ DM); Acid detergent fiber (ADF, g kg⁻¹ DM) and Acid detergent lignin (ADL, g kg⁻¹ DM) by the Van Soest and Robertson (1979) detergent assay and *in vitro* dry matter digestibility (IVDMD) according to a two-way pepsin-cellulase method of Aufrère (1982). The polyosides

are empirically calculated: Hemicellulose (g kg⁻¹ DM) = NDF – ADF and Cellulose (g kg⁻¹ DM) = ADF – ADL. The lignification degree is expressed as the percentage of ADL and NDF.

The nutritional value of the feed has been estimated by the Bulgarian system as Feed Unit for Milk (FUM, in kg DM) and Feed units for growth (FUG, in kg DM), and calculated on the basis of equations according to the experimental values of CP, CF, Cft and NFE, recalculated by Todorov digestibility ratios (2010): Gross energy (GE, MJ/kg DM) = 0.0242*CP + 0.0366*Cft + 0.0209*CF + 0.017*NFE – 0.0007*Zx and Exchangeable energy (EE, MJ/kg DM) = 0.0152*DP (Digestible protein) + 0.0342*Dft (Digestible fat) + 0.0128*DF (Digestible fibers) + 0.0159*DNFE (Digestible Nitrogen-free extractable substances) – 0.0007*Zx.

The content of plastid pigments (Chlorophyll „a“, Chlorophyll „b“, Carotenoids „c“ and Total) in fresh plant samples from the first growth was determined, according to the method of Zelenskii and Mogileva (1980). The relationships were empirically calculated:

- Chlorophyll „a“ / Chlorophyll „b“;
- Chlorophyll a + b / Carotenoids „c“.

Experimental data were averaged and statistically processed using a variance analysis ANOVA, LSD at P = 0.05.

Results and Discussion

The structural fibre components of cell walls are a determining factor in the nutritive value of forages. The fiber composition of the plant cell (neutral-detergent fiber, acid-

detergent fiber, acid-detergent lignin, hemicellulose, and cellulose) is one of the most important indicators that is often used as a predictor of digestibility or the amount of net energy in the forage.

Neutral-detergent fibres

Neutral-detergent fibre content significantly affects forage intake in ruminants (Fustini et al., 2017). In this aspect, in the first (571.8 g kg⁻¹ DM) and fourth experimental years (679.5 g kg⁻¹ DM), the grasses of *Dactylis glomerata* L. had the highest concentration of neutral-detergent fibre (NDF) (Figure 1). In the second (639.2 g kg⁻¹ DM) and third (663.1 g kg⁻¹ DM) growing seasons, as well as in the period average (634.0 g kg⁻¹ DM), the forage with the maximum values of the indicator was the dry matter of *Festuca rubra* L. For the entire study period, the forage of *Lolium perenne* L. had the lowest NDF content in dry matter (554.8 g kg⁻¹ DM). The difference in the amount of NDF determining forage uptake in the studied perennial forage grasses was significant ($P < 0.05$ and $P = 0.05$).

Acid-detergent fibres

There was no significant difference between the perennial forage grasses included in the experiment in terms of acid-detergent fibre content. Considering the influence of the lignocellulosic fraction on the digestibility of the forage, their reduced concentration is essential for forage quality. In this matter, *Lolium perenne* L. (319.7 g kg⁻¹ DM) had the lowest average values of the indicator, followed by *Dactylis glomerata* L. (329.1 g kg⁻¹ DM). The species *Festuca rubra* L. and *Phleum pratense* L. recorded higher ADF contents of 3.2 to 5.8% and 4.7 to 7.4%, respectively. The results indicate a 49.6% lower content of ADF in the dry matter of the studied crops compared to that found by Cerempei et al. (2022), where the mean value of the parameter was 499.0 g/kg DM. The increased fiber content may be due to plant species, maturity and environmental conditions (Mahmoud et al., 2017).

Acid-detergent lignin

Acid-detergent lignin, as part of the fibre composition of the cell walls, affects the digestibility of the plant mass inversely. On average over the period, the forage mass of *Lolium perenne* L. had the lowest content of ADL (41.1 g kg⁻¹ DM). In the conditions of the Middle Balkan Mountain, the plant mass of *Festuca rubra* L. (64.1 g kg⁻¹ DM) had the highest values of lignin in dry matter. The variation in the amount of acid-detergent lignin was most pronounced in the variants with *Dactylis glomerata* L., where the difference in trait values (by year) ranged from 8.7 to 18.2 g kg⁻¹ DM. The natural poly-

mer studied is a determinant of lodging resistance in grassland stands, and the data from the analysis indicate that its values are significantly correlated with environmental conditions (temperature and rainfall) and age of the grassland ($P < 0.01$). Acid-detergent lignin content was found to be lower in *Dactylis glomerata* L., *Festuca arundinacea* Schreb., *Festuca pratensis* L. and *Festuca rubra* L. crops grown under lowland conditions. The mean values of the indicator by subgrowth (first, second and third) in the variants with *Dactylis glomerata* L. were 4.66%, 3.94% and 3.16%, respectively, and for the genus *Festuca*: 3.17%, 3.51% and 2.60%, respectively (Katova and Naydenova, 2017a, b).

Hemicellulose

The biological completeness of forage depends largely on the content of the fully digestible polysaccharide hemicellulose and the incompletely digestible cellulose. Hemicellulose is a polysaccharide with relevance to the degree of digestibility and digestibility of forage by ruminants. The biomass of *Festuca rubra* L. and *Lolium perenne* L. had elevated levels (from the first to the fourth experimental year), and a significant superiority to the other species in the difference of trait values (Figure 2). On average over the period, the hemicellulose content of the plant mass of *Festuca rubra* L. (294.0 g kg⁻¹ DM) was shown to be higher compared to that of *Dactylis glomerata* L. (259.9 g kg⁻¹ DM), *Phleum pratense* L. (235.6 g kg⁻¹ DM) and *Lolium perenne* L. (235.1 g kg⁻¹ DM). The exceedance of the indicator values ranged from 11.6% to 20.0%. Over the study period, the sources of variation (years and crop type) significantly influenced the amount of hemicellulose ($P < 0.001$).

Cellulose

Cellulose, as a partially digestible and partially indigestible component influences the digestibility of dry matter the most, on which the nutritive value of forage depends (Naydenova, 2009). No significant difference was found in the mean values for the content of this fibre component in the perennial grass species studied. Compared to other crops, with increasing age of the stand, the amount of cellulose in the dry matter of *Dactylis glomerata* L. followed an increasing trend, despite the fact that the plant mass had the lowest values of the indicator in the first (257.4 g kg⁻¹ DM), second (249.5 g kg⁻¹ DM), third vegetation (263.6 g kg⁻¹ DM) and average for the experiment (269.7 g kg⁻¹ DM).

For the experimental period, *Phleum pratense* L. grasslands had the highest cellulose content (288.5 g kg⁻¹ DM). Data from the analysis showed that, over the years of study, the cellulose content varied most in the dry matter of *Festuca rubra* L. (up to 22.8%).

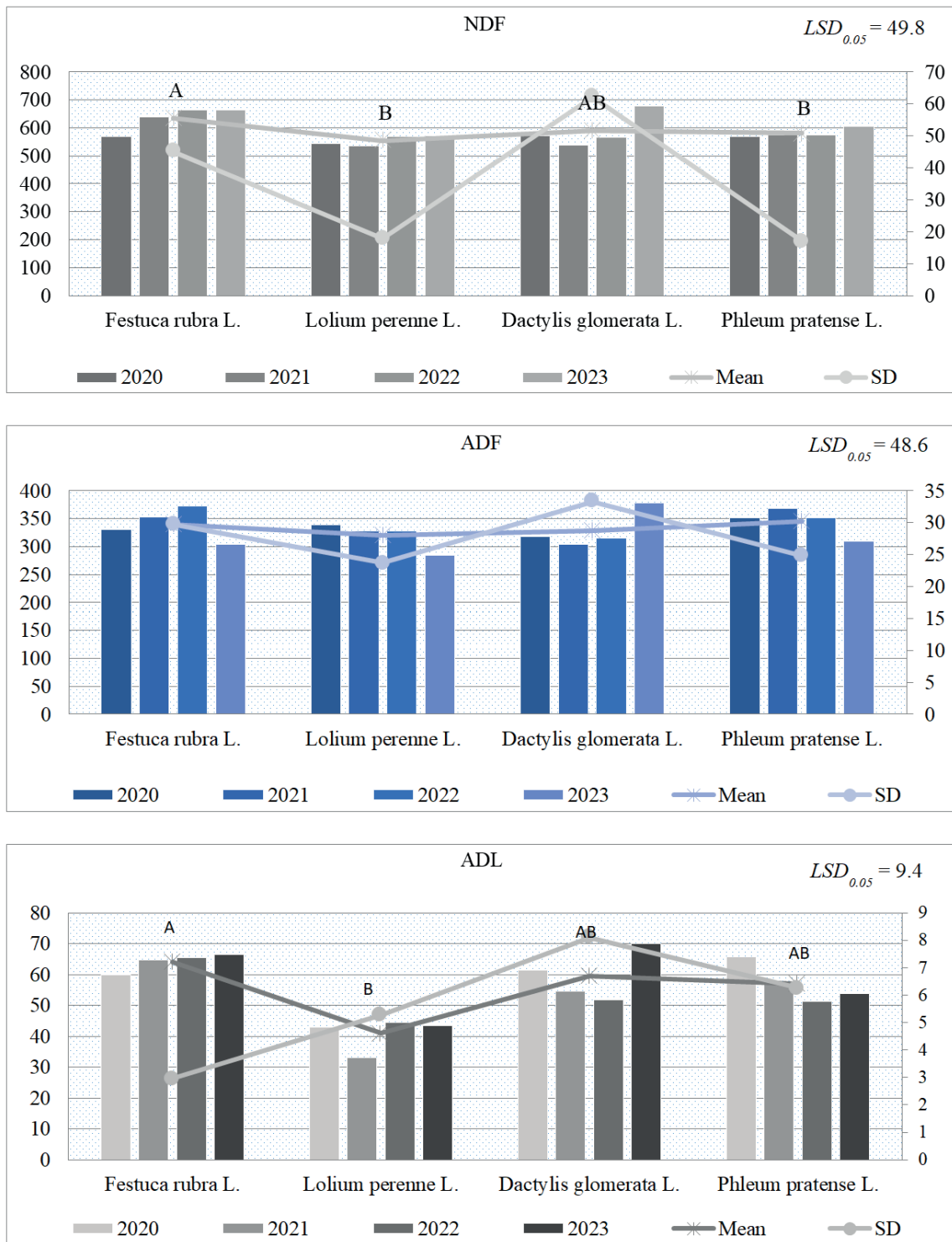


Fig. 1. Content of neutral-detergent fibres, acid-detergent fibres and acid-detergent lignin (g kg⁻¹ DM) in dry matter of perennial grasses, by year and average for the period

Source: Authors' own elaboration

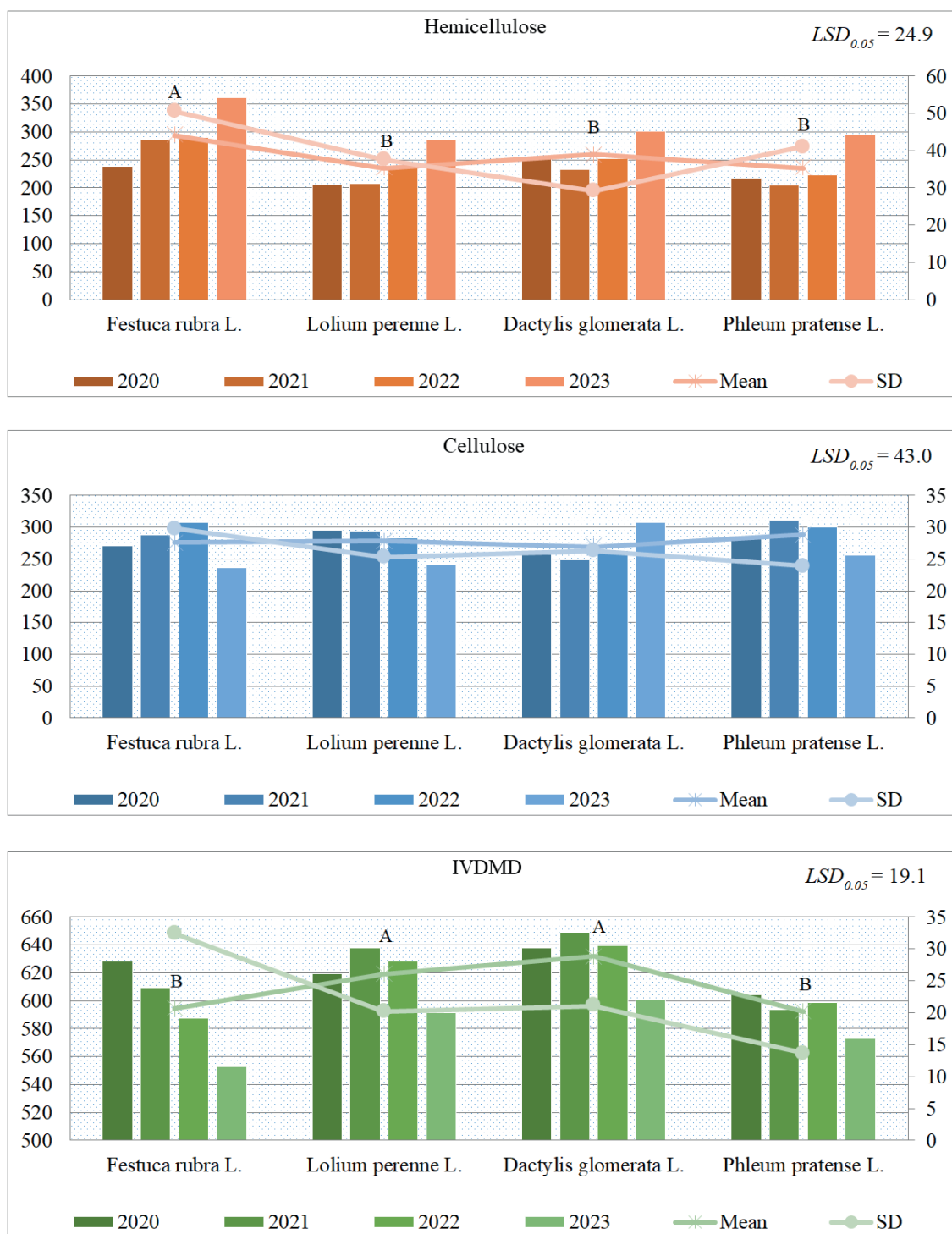


Fig. 2. Content of hemicellulose, cellulose and *in vitro* digestibility of dry matter ($g\ kg^{-1}\ DM$) in perennial grasses, by year and average for the period
 Source: Authors' own elaboration

In vitro dry matter digestibility

Changes in the fibre composition of the plant mass were attributed to seasonal variations in the region, as well as to botanical features in the perennial grass species studied, related to the accumulation of the fibre fraction and its influence on the quality and digestibility of the dry matter. For the four-year test period, the biomass with the highest *in vitro* dry matter digestibility was that of *Dactylis glomerata* L. (631.8 g kg⁻¹ DM) and *Lolium perenne* L. (619.3 g kg⁻¹ DM). Orchardgrass (*Dactylis glomerata* L.) is one of the most tolerant grass species (considering soil and climatic conditions) in Bulgaria, whose digestibility ranges from 59.10% to 65.98% (Naydenova et al., 2015). Leaves and stems of perennial ryegrass (*Lolium perenne* L.) are significantly more digestible than those of other grass species, which determines the relatively high digestibility of total forage (50.95–72.90%) (Naydenova and Katova, 2014).

In the conditions of the Middle Balkan Mountains, the species *Festuca rubra* L. and *Phleum pratense* L. recorded up to 6.3% and 6.6% significantly lower dry matter digestibility compared to the average maximum value of the indicator. The results of the analysis of variance for the factorial influence on *in vitro* dry matter digestibility showed a high degree of confidence regarding the observed differences. The values of the indicator were significantly influenced by the

environmental conditions in the growing area as well as by the type of perennial grasses ($P < 0.01$).

Degree of lignification

Cell wall lignification creates an access barrier to potentially digestible wall material by rumen bacteria if cells have not been physically ruptured (Junga et al., 2012). There was a significant difference in the coefficients of degree of lignification in the forage mass of the forage species studied (Figure 3).

In the years of the experiment (coefficient = 6.2–7.9) and on average over the period (coefficient = 7.4), the lowest degree of lignification was observed in the pure grasslands of *Lolium perenne* L.

The exceedance of the indicator values in the variants of *Festuca rubra* L. (coefficient = 10.1), *Dactylis glomerata* L. (coefficient = 10.1) and *Phleum pratense* L. (coefficient = 9.8) ranged from 24.5% to 26.7%. Environmental conditions demonstrably influenced ($P < 0.001$) the degree of lignification among the treatments included in the experiment.

Nutritional energy value of forage

Accurate establishment of energy nutritive value is a key criterion for modern forage quality assessment and is determined by milk and growth units (Todorov, 2010).

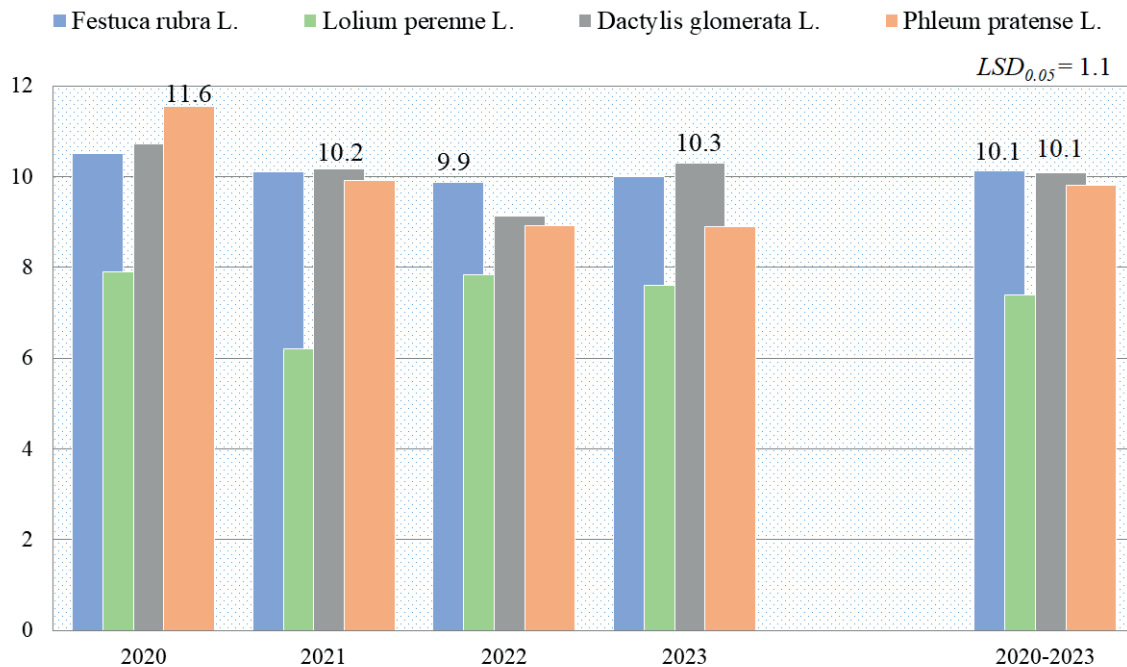


Fig. 3. Degree of lignification (coefficient) of perennial forage grasses, average over the period

Source: Authors' own elaboration

For the experimental period, the amount of gross energy in the biomass of perennial meadow grasses ranged from 18.52 MJ/kg to 18.77 MJ/kg (Table 3). The dry mass of *Phleum pratense* L. and *Dactylis glomerata* L. was higher than the average value (18.65 MJ/kg). According to Naydenova et al. (2015), Naydenova and Vasileva (2016), Katova (2023b), *Dactylis glomerata* L. is a high energy forage crop with a wide distribution range in the temperate climate of Bulgaria and good forage quality indicators. *Phleum pratense* L. is a species that exhibits sensitivity to prolonged drought and high temperatures, because of its root system poor suction power (Jargiello and Harrot, 1989). In this case, data analysis indicated that the potential energy-nutrient value of *Phleum pratense* L. (under upland conditions) was not significantly affected by differences in climatic conditions over the years of study. The recollected forage mass

Table 3. Potential energy nutrient value of perennial grasses, by year and average for the period

Variants	GE	EE	FUM	FUG
2020				
<i>Festuca rubra</i> L.	18.03	8.08	0.75	0.68
<i>Lolium perenne</i> L.	18.72	7.69	0.70	0.62
<i>Dactylis glomerata</i> L.	18.50	7.84	0.72	0.65
<i>Phleum pratense</i> L.	18.69	7.76	0.71	0.63
2021				
<i>Festuca rubra</i> L.	18.72	7.81	0.71	0.64
<i>Lolium perenne</i> L.	18.59	7.23	0.65	0.57
<i>Dactylis glomerata</i> L.	18.83	7.76	0.71	0.63
<i>Phleum pratense</i> L.	18.59	7.38	0.67	0.59
2022				
<i>Festuca rubra</i> L.	18.60	7.38	0.67	0.59
<i>Lolium perenne</i> L.	18.49	7.61	0.69	0.62
<i>Dactylis glomerata</i> L.	18.87	7.60	0.69	0.61
<i>Phleum pratense</i> L.	18.93	7.89	0.72	0.64
2023				
<i>Festuca rubra</i> L.	18.72	7.59	0.69	0.61
<i>Lolium perenne</i> L.	18.46	7.68	0.70	0.63
<i>Dactylis glomerata</i> L.	18.80	7.62	0.69	0.61
<i>Phleum pratense</i> L.	18.87	7.77	0.71	0.63
2020–2023				
<i>Festuca rubra</i> L.	18.52	7.71	0.70	0.63
<i>Lolium perenne</i> L.	18.56	7.55	0.69	0.61
<i>Dactylis glomerata</i> L.	18.75	7.71	0.70	0.63
<i>Phleum pratense</i> L.	18.77	7.70	0.70	0.62
Mean	18.65	7.67	0.70	0.62
SD	0.13	0.08	0.01	0.01

Legend: GE (MJ/kg DM), EE (MJ/kg DM), FUM (in kg DM), FUG (in kg DM).

Source: Authors' own elaboration

was also without significant difference in the values of empirically calculated indices – GE, EE, FUM and FUG in the vegetation years with the lowest and highest annual rainfall, respectively.

Concerning the amount of energy that regulates metabolic processes (EE) in the animal organism, monoculture crops of *Festuca rubra* L., *Dactylis glomerata* L. and *Phleum pratense* L. recorded 0.4–0.5% higher trait values compared to the average for the study period (7.67 MJ/kg). Because forages can be digested by ruminants at a certain level, their metabolizable energy contents are very important (Colf, 2010).

The crude unit content gives an idea of the energy value or nutritive value of the forage, but animal production also depends on the amount of forage ingested or the amount of net energy absorbed (FUM and FUG). In the years of the experiment, the values of FUM and FUG in the studied forage ranged from 0.65 counts in kg DM in *Lolium perenne* L. to 0.75 counts in kg DM in *Festuca rubra* L. (with an average value of 0.70 counts in kg DM) and from 0.57 counts in kg DM (*Lolium perenne* L.) to 0.68 counts in kg DM (*Festuca rubra* L.) (with an average value of 0.62 counts in kg DM), respectively.

Plastid pigments

The chlorophyll in plants uses the intensity of the sun to store energy in the form of carbohydrates. The concentration of plastid pigments in the fresh matter of perennial grasses is influenced by age, seasonal changes and plant type (Katova et al., 2018).

The content of the main photosynthetic pigments in the leaves of the studied forage crops varied from 92.51 mg/100 g FW to 126.48 mg/100 g FW (chlorophyll „a“) and from 65.88 mg/100 g FW to 107.78 mg/100 g FW (chlorophyll „b“) – (Table 4). The species with the highest and respectively the lowest mean values of the parameters were *Lolium perenne* L. and *Dactylis glomerata* L.

The role of chlorophyll „a“ is vital in the conversion of light energy into chemical energy, which leads to the synthesis of substances involved in the construction of sugars at a subsequent stage in the dark phase of photosynthesis to create organic compounds (Faic, 2018; Stoyanova et al., 2019). This implies a change in the composition of the carbohydrate fraction in the dry matter of perennial grass species, as well as in the palatability of the forage mass. With a higher chlorophyll „a“ content compared to the average value of the indicator (107.31 mg/100 g FW) was the foliage of *Lolium perenne* L. (by 17.9%) and *Festuca rubra* L. (by 0.5%).

Chlorophyll „b“ and β -carotenoids are so-called „additive pigments“ that allow plants to capture and use more sunlight. In the light of the results obtained, the higher concen-

Table 4. Content of plastid pigments in leaves of perennial grasses (mg/100 g FW)

Variants	Chlorophyll „a”	Chlorophyll „b”	Carotenoids „c”	a:b	a+b / c	Total
<i>Festuca rubra</i> L.	107.86	78.75	38.16	1.37	4.89	224.78
<i>Lolium perenne</i> L.	126.48	107.78	45.96	1.17	5.10	280.21
<i>Dactylis glomerata</i> L.	92.51	65.88	34.41	1.40	4.60	192.80
<i>Phleum pratense</i> L.	102.40	78.79	38.69	1.30	4.68	219.88
Mean	107.31	82.80	39.30	1.31	4.82	229.42
CV, %	13.30	21.41	12.28	7.77	4.60	15.98

Source: Authors' own elaboration

tration of chlorophyll „b” in the leaves of *Lolium perenne* L. gives rise to a higher efficiency of the photosynthetic process, respectively, a more optimal accumulation of nutrients in the plant mass. The excess of the indicator values over the average (82.80 mg/100 g FW) is was 32.2%.

Carotenoids, as part of the photosynthetic pigments in the plant cell, have a protective function regarding the absorption of excess solar energy and its conversion into heat. The carotenoid content of different perennial grass species varies from 34.41 mg/100 g FW to 45.96 mg/100 g FW with an average value of 39.30 mg/100 g FW. The plastid pigment content gives a clear picture of the physiological changes that occur in plant metabolism especially when water balance is disturbed (Velinova and Naydenova, 2008).

The ratios: chlorophyll „a” / chlorophyll „b” and chlorophyll a+b / c were relatively constant values, ranging in a narrow range from 1.17 mg/100 g FW to 1.40 mg/100 g FW and from 4.60 mg/100 g FW to 5.10 mg/100 g FW, respectively. The values of these parameters are related to the functional activity of the photosynthetic apparatus, which is particularly sensitive to environmental changes, response mechanisms to stress factors and plant cell senescence.

The green pigment (chlorophyll) has a significant influence on the nutritive value of the plant mass, and high total plant pigment content is one of the main factors stimulating biological productivity of plants (Kostadinov and Kostova, 2013). According to the data we obtained, the plants with the maximum values in total plastid pigment content were the stand-alone crops of *Lolium perenne* L. (280.21 mg/100 g FW). The excess in the values of the indicator compared to the average is was 22.1%.

Conclusions

Analysis of the structural fibre components of the cell walls showed that the forage of *Lolium perenne* L. had the lowest content of neutral-detergent fibres, acid-detergent fibres and acid-detergent lignin, while *Festuca rubra* L. grasses were shown to have the highest hemicellulose content.

The biomass of *Dactylis glomerata* L. had the highest *in*

vitro dry matter digestibility (631.8 g kg⁻¹ DM). It exceeded the average value by 3.7%. For the experimental period, the harvested biomass also had the lowest cellulose content.

The grasslands of *Phleum pratense* L. and *Dactylis glomerata* L. had the highest energy-nutrient value, and the fresh leaf mass of *Lolium perenne* L. had the highest total plastid pigment content (280.21 mg/100 g FW).

References

- Akalin, M. (2014). Impacts of climate change on agriculture: Adaptation and mitigation strategies to address these effects. *J. Hitit Univ. Inst. Soc. Sci.*, 7, 351 – 377.
- Aufrère, J. (1982). Study of the forecast of the digestibility of forages by an enzymatic method. *Annales de Zootechnie*, 31(2), 111 – 130.
- Billen, G., Le Noe, J. & Garnier, J. (2018). Two contrasted future scenarios for the French agro-food system. *Sci. Total Environ.*, 637-638, 695 – 705. <https://doi.org/10.1016/j.scitotenv.2018.05.043>.
- Bozhanska T., Petkova, M., Iliev, M., Georgieva, M., Georgiev, D. & D. Hristova, (2024). Botanical and basic chemical composition of forage from perennial grass crops grown in monoculture and mixed grassland under mountain conditions. *Scientific Papers. Series A. Agronomy, LXVIII*(1), 286 – 295.
- Bozhanska, T. & Churkova, B. (2019). Growth and development of legume and grass components in mixed grasslands grown in the Central Balkan mountain. *Trakia Journal of Sciences*, 17(1), 19 – 27.
- Bozhanska, T. (2017). Productivity and quality characteristic of forage grasses and grass mixtures for the conditions of the Central Balkan Mountain. Dissertation, PhD, 196 p. (Bg).
- Cerempei V., Țiței, V., Blaj, V., Andreoiu, A., Marușca, T., Mazare, V., Doroftei, V., Ababii A. & Guțu, A. (2022). The physical properties of seeds and the biochemical composition of the straw of romanian *Festuca* cultivars grown under the conditions of the Republic of Moldova. *Lucrări Științifice, seria Agronomie*, 65(2), 75 – 80.
- Colf, J. V. D. (2010). The production potential of Kikuyu (*Pennisetum clandestinum*) pastures over-sown with ryegrass (*Lolium* spp.). Master Thesis Study, University of Pretoria, 42 – 67.
- Churkova, K. & Churkova, B. (2024a). Productive capacities and adaptability of meadow legume crops under mountain conditions. *Bulgarian Journal of Agricultural Science*, 30(3), 503 – 508.

- Churkova, K. & Churkova, B.** (2024b). Status and Opportunities of Natural Grasslands in Forage Production. *Journal of Mountain Agriculture on the Balkans*, 27(1), 157 – 175.
- Faic, A. K.** (2018). Mechanisms of response of photosynthetic apparatus of higher plants to combined temperature and light stress. Abstract of dissertation for the award of the scientific and educational degree “Doctor”, Institute of Biophysics and Biomedical Engineering – BAS (Bulgaria), 44.
- Fustini M., Palmonari, A., Canestrari, G., Bonfante, E., Mami, L., Pacchioli, M. T., Sniffen, G. C. J., Grant, Cotanch, R. J., K. W. & Formigoni, A.** (2017). Effect of undigested neutral detergent fiber content of alfalfa hay on lactating dairy cows: Feeding behavior, fiber digestibility, and lactation performance. *J. Dairy Sci.*, 100, 4475 – 4483. <https://doi.org/10.3168/jds.2016-12266>.
- Jargiello, J. & Harkot, W.** (1989). Effect of soil moisture on the growth and development of seedlings of some grass species. 16th General Meeting of the European Grassland Federation, Grado, Italy. Academy of Agriculture, Lublin, Poland, 90 – 934.
- Junga, G.H-J, Samac, D. A. & Sarath, G.** (2012). Modifying crops to increase cell wall digestibility. *Plant Science*, 185-186, 65 – 67.
- Katova, A. & Naydenova, Y.** (2017a). Chemical composition, digestibility and feeding value of accessions from genus *Festuca*. *Journal of Mountain Agriculture on the Balkans*, 20(3), 16 – 34.
- Katova, A. & Naydenova, Y.** (2017b). Chemical composition, digestibility and feeding value of accessions orchardgrass (*Dactylis glomerata* L.). *Journal of Mountain Agriculture on the Balkans*, 20(3), 1 – 15.
- Katova, A.** (2023a). Breeding assessment of leafiness for varieties and ecotypes of cocksfoot (*Dactylis glomerata* L.). *Journal of Mountain Agriculture on the Balkans*, 26(1), 210 – 227.
- Katova, A.** (2023b). Breeding assessment of leafiness for species, varieties and ecotypes of genus *Festuca*. *Journal of Mountain Agriculture on the Balkans*, 26(2), 141 – 161.
- Katova, A. Vulchinkov, J. & Ilieva, A.** (2018). Breeding assessment by photosynthetic pigments content of fescue (*Festuca ssp.*) and cocksfoot (*Dactylis glomerata* L.) accessions. *Field Crops Studies*, XI(2), 53 – 68.
- Khan, M. N., Ali, S., Yaseen, T., Ullah, S., Zaman, A., Iqbal, M. & Shah, S.** (2019). Eco-Taxonomic Study of Family Poaceae (Gramineae), RADS. *Biol. Res. Appl. Sci.*, 10(2), 63 – 75.
- Kosolapov, V. M., Kostenko, S. I., Tyurin, Yu S., Shamsutdinova, E. Z. & Piskovskii, Y. M.** (2021). Perennial forage grasses – the basis for greening agricultural production. Economic and Phytosanitary Rationale for the Introduction of Feed Plants, IOP Conf. Series: Earth and Environmental Science, 663, 012022. doi:10.1088/1755-1315/663/1/012022.
- Kostadinov, K. & Kostova, D.** (2013). Influence between the content of zinc in leaves and the biological manifestations of eggplant (*Solanum melongena* L.) in foliar spray. *Plant Science*, 50, 58 – 63.
- Kostov, K. & Pavlov, D.** (1999). Forage production. Academic Publishing House of Higher Agricultural Institute, Plovdiv, Bulgaria, 223.
- Mahmoud A. E. M., Abbas, M. S., Cieslak, A. & Szumacher-Strabel, M.** (2017). Evaluation of chemical composition and in vitro dry and organic matter digestibility of some forage plant species derived from Egyptian rangelands. *The Journal of Animal & Plant Sciences*, 27(5), 1573 – 1581.
- Mitev, D. & Naydenova, G.** (2014). Permanence of sown sward situated along the slopes of the Central Balkan mountain. *Biotechnology in Animal Husbandry*, 30(3), 509 – 516.
- Naydenova, Y. & Katova, A.** (2014). Forage quality evaluation of diploid perennial ryegrass (*Lolium perenne* L.) in competitive variety trial. *Journal of Mountain Agriculture on the Balkans*, 17(6), 1665 – 1677.
- Naydenova Y. & Katova, A.** (2015). Feeding Value Estimation of Perennial Ryegrass, Alfalfa and Their Mixtures. *Plant Science*, LII(5), 106 – 113.
- Naydenova Y., Vasilev, E. & Kirilov, A.** (2015). Plant cell wall fiber components content and digestibility of orchardgrass (*Dactylis glomerata* L.) and legume forage species in pure stands and mixtures. *Journal of Mountain Agriculture on the Balkans*, 18(1), 61 – 76.
- Naydenova, Y. & Vasileva, V.** (2016). Analysis of Forage Quality of Grass Mixtures – Perennial Grasses with Subterranean Clover. *Journal of Basic And Applied Research*, 2(4), 534 – 540.
- Naydenova, Y.** (2009). Forage quality analysis and evaluation in the breeding process of perennial grasses. *Field Crops Studies*, V(2), 357 – 375.
- Nazli, R. I., Kuvuran, Al., Tansi, V., Ozturk, H. H. & Budak, D. B.** (2020). Comparison of cool and warm season perennial grasses for biomass yield, quality, and energy balance in two contrasting semiarid environments. *Biomass and Bioenergy*, 139, 105627. <https://doi.org/10.1016/j.biombioe.2020.105627>.
- Ninov, N.** (1997). Geography of Bulgaria. Professor Marin Drinov Academic Publishing House. Sofia, 240 – 260.
- Penkov, M.** (1988). Soil Science. *Zemizdat*, Sofia, 179 – 190.
- Perotti E., Huguenin-Elie, O., Meisser, M., Dubois, S., Probo, M. & Mariotte, P.** (2021). Climatic, soil, and vegetation drivers of forage yield and quality differ across the first three growth cycles of intensively managed permanent grasslands. *European Journal of Agronomy*, 122, 126194. <https://doi.org/10.1016/j.eja.2020.126194>.
- Petkova, M. & Iliev M.** (2025). Longevity of Self-Seeded and Mixed Grasslands of Pasture Ryegrass and Legume Meadow Grasses in the Central Balkan Mountains. *Journal of Mountain Agriculture on the Balkans*, 28(2), 217 – 240. <https://jmbonline.com/bg/article/TMYilgekjIXq1X4zw9Sh>
- Rivero, M. J., Balocchi, O. A., Moscoso, C. J., Siebald, J. A., Neumann, F. L., Meyer, D. & Lee, M. R. F.** (2019). Does the “high sugar” trait of perennial ryegrass cultivars express under temperate climate conditions? *Grass Forage Sci.*, 74, 496 – 508. <https://doi.org/10.1111/gfs.12406>.
- Robins, J. G., Waldron, B. L. & Jensen, K. B.** (2020). Productivity, stability, and resilience of cool-season perennial grasses used for rangeland revegetation. *Agrosystems, Geosciences & Environment*, 3(1), 1 – 7. <https://doi.org/10.1002/agg2.20002>.
- Rusinovci, I., Fetahu, S., Zeka, D., Bytyqi, H. & Aliu, S.** (2016). Yield and quality traits of some forage crops cultivated under agroecological conditions of Kosova. *Agriculture & Forestry*, 62(2), 111 – 118.
- Savev, L. & Stanev, S.** (1963). Climatic regions of Bulgaria and

- their climate. *Zemizdat Press*, Sofia, 180 (Bg).
- Scordia, D. & Cosentino, S. L.** (2019). Perennial energy grasses: Resilient crops in a changing European agriculture. *Agriculture*, 9, 169. doi:10.3390/agriculture9080169.
- Severoglu, S. & Gullap, M. K.** (2023). Determination of Feed Yield and Quality Parameters of Bermudagrass (*Cynodon dactylon* L. (Pers.)) Populations Collected from Natural Flora. *Agronomy*, 13, 1471. <https://doi.org/10.3390/agronomy13061471>.
- Stoyanova, S., Petrova, I. & Marinova, D.** (2019). Performance of productive potential of winter rape (*Brassica napus*) at different sowing times. *Journal of Mountain Agriculture on the Balkans*, 22(4), 122 – 135.
- Tenikecier, H. S. & Ates, E.** (2018). Chemical Composition of Six Grass Species (*Poaceae* sp.) from Protected Forest Range in Northern Bulgaria. *Asian Journal of Applied Sciences*, 11(2), 71 – 75.
- Țiței, V.** (2018). The quality of preserved biomass of some *Poaceae* species under the conditions of Republic of Moldova. *Lucrări Științifice*, 61(1), 177 – 182.
- Todorov, N.** (2010). Practice in Animal Nutrition. *East West*, Sofia, 462.
- Van Soest, P. J. & Robertson, J. B.** (1979). Systems of analysis evaluating fibrous feeds. Cornell University-Ithaca, N.Y.
- Velinova, K. & Naydenova, Ts.** (2008). Contents of pigments, total protein and free proline in the assimilating apparatus of scots pine (*Pinus sylvestris* L.) and austrian black pine (*Pinus nigra* Arn.) in different soil moisture. *Forest Science*, 1, 3 – 15.
- Zelenskii, M. & Mogileva, G.** (1980). Comparative Evaluation of Photosynthetic Ability of Agricultural Crops by Photochemical Activity of Chloroplasts. *VIR*, Leningrad, 36 (Ru).

Received: August, 26, 2024; Approved: December, 30, 2024; Published: April, 2026