

**BOTANICAL COMPOSITION AND QUALITY OF FORAGE  
IN SELF-SEEDED GRASSLANDS OF *FESTULOLIUM*  
IN MOUNTAIN CONDITIONS**

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**Abstract**

The aim of the study is to track changes in the composition and quality of forage biomass from *Festulolium* cultivars (Hykor, Lofa, Felina, and Perun) in the foothills of the Central Balkan Mountains (Bulgaria).

*Festulolium* cultivars show high resistance, persistence, and competitiveness in medium- to long-term cultivation in mountain conditions. A clear dynamic has been observed in the changes in the relative share of the main species and the degree of weed infestation in the formed above-ground mass. In the year of sowing, the presence of *Festulolium* in the sown grasslands varies from 13.5 to 52.4%, and in the second, third, and fourth experimental years – from 75.7 to 99.1%. The crude protein content in the dry matter of the studied *Festulolium* cultivars ranged from 79.73 g kg<sup>-1</sup> DM (cv. Lofa) to 92.88 g kg<sup>-1</sup> DM (Perun). The Lofa cultivar is distinguished by the highest content of crude fat, minerals, and calcium and the lowest concentration of the macroelement P (from 35.00 to 119.29% compared to the other *Festulolium* cultivars studied). The highest and lowest in vitro dry matter digestibility values were observed in the Perun (636.11 g kg<sup>-1</sup> DM) and Felina (605.38 g kg<sup>-1</sup> DM) cultivars, respectively. The amount of crude fibre is highest in the self-seeded grasslands of the Hykor cultivars (381.50 g kg<sup>-1</sup> DM). The forage mass of this cultivar also has the lowest mineral content (60.00 g kg<sup>-1</sup> DM). The degree and significance of the factorial effects on the changes in the basic chemical and botanical composition of the studied *Festulolium* cultivars are reliable ( $P < 0.01$  –  $P < 0.001$ )

and can optimize cultivar selection and ecosystem management in mountainous regions.

**Key words:** *Festulolium*, floristic composition, crude protein, crude fibre, nitrogen-free extractable substances

**Introduction.** Global climate change requires the implementation of a well-founded and targeted policy to mitigate adverse impact on the production of high-quality grass forage in mountainous regions. In this context, interest is focused on the cultivation of interspecific hybrid forage grasses with high productive potential of good quality, and resistance to unfavourable habitat conditions [1, 2].

Chromosomal introgressions in the grass species *Festuca* (fescue) and *Lolium* (ryegrass) are used to produce *Festulolium* spp. hybrids, which, by introducing beneficial traits from one species into another, exhibit complementary characteristics [3, 4]. The above-ground mass of *Festuloliums* possesses a complex of positive agronomic traits of the parental forms, such as: high dry matter content, rapid growth, resistance to repeated mowing and grazing, tolerance to abiotic stress (especially drought), and high nutritional value, which makes it unique in the creation and preservation of new cultivars [5, 6]. The growth and development of the hybrid germplasm begins early in spring. The plants are well leafed, show good winter and drought resistance, which makes them suitable for cultivation in mountainous and semi-mountainous areas [7]. The harvested grass mass has a higher crude protein content compared to pure *Festuca* species, but lower than *Lolium* species [8]. The *Festulolium* genome is characterized by a relatively high content of water-soluble carbohydrates (WSC), which has a positive effect on the digestibility of dry matter and at the same time decreased at successive harvests [9].

Studies related to agronomic characteristics, forage value, and the creation of new cultivars are increasing interest in *Festulolium* species in terms of their regional adaptability and application. The aim of this study is to track changes in the composition and quality of forage biomass from *Festulolium* cultivars in the foothills of the Central Balkan Mountains (Bulgaria).

**Material and methods.** At the experimental field of the Research Institute of Mountain Stockbreeding and Agriculture, Troyan (Bulgaria), during the period 2020–2023, we monitored the development of four *Festulolium* cultivars (Hykor – CZ, EE, FI, NO; Lofa – EE, RS; Felina – CZ and Perun – CZ, EE, RS) grown in separate grasslands, in four replicates, with a harvest plot size of 5 m<sup>2</sup>.

The field experiment was conducted on light gray, pseudopodzolic soils with pH = 4.2–5.5. The seeds were sown manually (scattered) in spring (March 10–15) at a rate consistent with that of the species in its pure state (3.5 kg/dka). In the year of sowing, we conducted two sanitary movings to reduce weed vegetation. The grasslands were mowed in the heading/flowering phase. The forage biomass was harvested twice over the course of the year.

The experimental territory belongs to the Pre-Balkan (mountain) climate region of the temperate-continental climate subregion. The average annual temperature is characterized by territorial differentiation (from north to south) with increasing altitude. The average annual temperatures are 10/11 °C. 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.

During the years of observation, we noted an increase in average air temperatures ranging from 0.3 °C to 1.6 °C, while the total precipitation during the growing season was 161.0 mm lower than the long-term average (Table 1).

T a b l e 1

Average weather conditions for March – October.  
In parentheses: the average norm (1990–2019)

	March	April	May	June	July	August	September	October
Temperature (°C)	5.4 (5.7)	9.5 (10.8)	15.1 (14.3)	18.9 (18.2)	22.1 (20.8)	22.0 (20.7)	17.3 (15.7)	12.2 (10.9)
Rainfall (mm)	35.3 (56.7)	65.0 (66.9)	87.5 (98.2)	101.3 (111.8)	37.7 (98.0)	56.9 (66.7)	33.2 (69.5)	48.0 (58.1)

Changes in meteorological conditions affected the composition of spring and summer growth, the time of harvesting, the amount of leaf and stem fraction, as well as the quantity and quality of the harvested forage mass.

The following indicators were monitored:

- Botanical composition of grassland (%) – determined by weight analysis of samples of green mass taken at each mowing of each variation. Their weighing was carried out in an air-dry state, by weighing the percentage of grass species.
- The chemical composition of dry feed was analyzed according to *Weende* analysis: Crude protein (CP, g kg<sup>-1</sup>) according to *Keldahl* (according to BDS/ISO-5983); Crude fibre (CFr, kg kg<sup>-1</sup>); Crude fat (CF, g kg<sup>-1</sup>) (according to BDS/ISO-6492); Crude ash (Ash, g kg<sup>-1</sup>) (according to BDS/ISO-5984); Dry matter (DM, g kg<sup>-1</sup>); Nitrogen-free extractable substances (NFE, %) = 100 – (CP, % + CFr, % + CF, % + Ash, % + Moisture, %) converted to g kg<sup>-1</sup>; calcium (Ca, g kg<sup>-1</sup>) – complexometric and phosphorus (P, g kg<sup>-1</sup>) – with vanadate-molybdate reagent according method and in vitro dry matter digestibility (IVDMD, g kg<sup>-1</sup>) according to two-step pepsin-cellulose method of AUFRERE [10].

The data were processed using ANOVA variance analysis. To evaluate the data on changes in the chemical composition and digestibility of the feed, arithmetic mean (Mean) and coefficient of variation (CV, %) were calculated.

**Results and discussion.** In the year of sowing, the cultivars Hykor, Felina, and Lofa have a higher degree of weed infestation and a lower presence of the main species in the grass stand (Fig. 1). The ratio of the cultivated and weed fractions is 13.5/86.5%, 15.4/84.6%, and 43.2/56.8%, respectively. The *Festulolium* species demonstrates a 2.4% predominance over the weed vegetation only in the grasslands of the Perun cultivars.

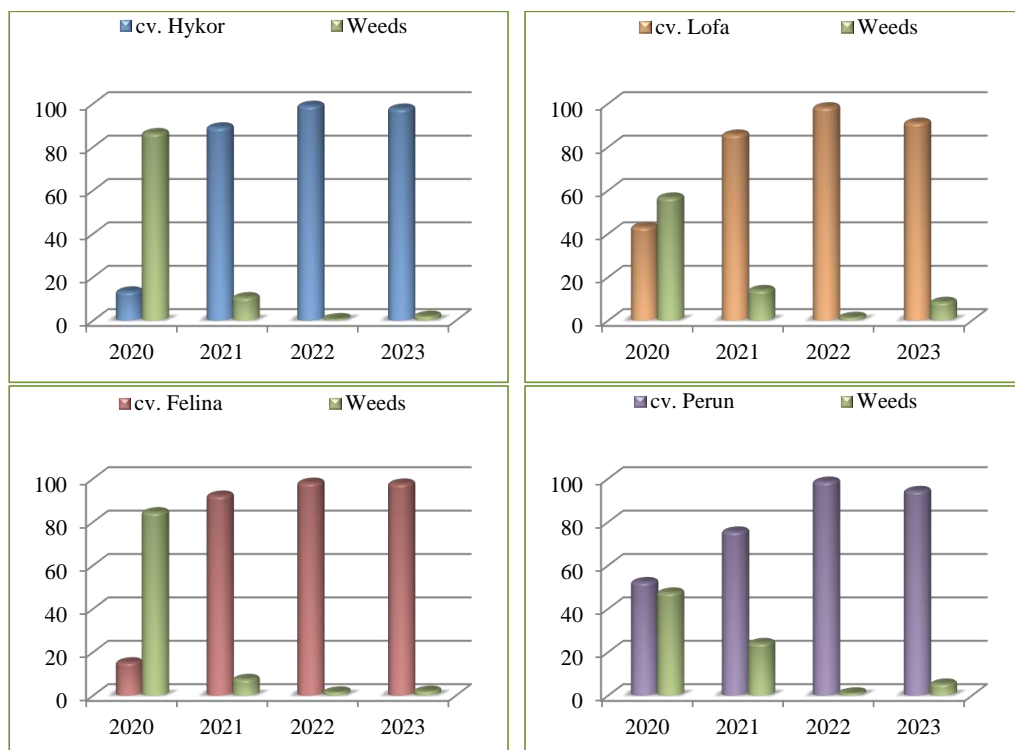


Fig. 1. Botanical composition of cultivars *Festulolium*, %

According to data from CZABAN et al. [11] and KOVÁCS et al. [12], *Festulolium* hybrids possess specific physiological and morphological characteristics of their parent forms (*Lolium* and *Festuca*), which determine their suitability and resistance. The data from the analysis show that in the second experimental year, the relative share of perennial crops in the formed grasslands is higher. The values of the indicator vary from 75.7% (cv. Perun) to 92.3% (cv. Felina). The cultivar with the lowest average annual fresh mass productivity in the summer growth (cv. Felina) recorded the highest presence in the grassland, which suggests good adaptability and resistance of the plants under the test conditions. According to

Ghesquière et al. [13], cultivars with slower growth and development (productivity, respectively) over the years maintain a higher presence in the grassland due to increased persistence and more limited use of soil resources.

During the third experimental year, *Festulolium* cultivars had the lowest weed infestation rate (0.9–1.8%) and the highest relative share of sown cereal grass (98.2–99.1%) in the above-ground biomass. In the fourth experimental year, a general downward trend was observed in the relative share of *Festulolium*, ranging from 0.4% (cv. Felina) to 7.1% (cv. Lofa) with weed infestation of 2.2–8.7%.

The observed dynamics in the changes in the relative share of the main species and the degree of weed infestation is comparable to data from foreign studies [2], according to which the *Festulolium* species is characterized by high adaptive potential, but with increasing age of the grass stand, there is a gradual reduction in the main species and a decrease in its regenerative potential.

**Chemical composition of *Festulolium* cultivars.** The high proportion of the species *Festulolium* has a positive effect on the forage value of the produced grass mass, while the protein fraction is an important factor in assessing the nutritional value of the forage.

During the years of the experiment, the crude protein content in the dry matter of the studied *Festulolium* cultivars was low. The mean values of this parameter ranged from 79.73 g kg<sup>-1</sup> DM (cv. Lofa) to 92.88 g kg<sup>-1</sup> DM (cv. Perun), which is 1.3–2.5 times lower than the level required for adequate nutrition of ruminant animals (Table 2). According to Wiśniewska-Kadžajan et al. [14], the crude protein content in the diets of dairy cows should exceed 150 g kg<sup>-1</sup> DM.

The Cfr content varies from 367.63 to 381.50 g kg<sup>-1</sup> DM. The fibre fraction in the composition of the Perun cultivar is up to 3.77% lower in concentration compared to the other *Festulolium* cultivars, which had a positive effect on the digestibility of the dry matter. The excess in IVDMD values is up to 5.08%.

T a b l e 2

Main chemical composition and in vitro digestibility of dry matter of *Festulolium* cultivars averaged over the period (g kg<sup>-1</sup> DM)

Cultivars	CP	CF	Cfr	Ash	NFE	Ca	P	IVDMD of Aufrère
Hykor	89.25	23.20	381.50	60.00	354.95	12.85	2.09	611.76
Lofa	79.73	23.63	377.88	80.93	344.78	17.25	1.40	633.33
Felina	91.08	18.43	374.85	65.38	360.35	10.65	3.07	605.38
Perun	92.88	20.65	367.63	65.70	359.85	13.95	1.89	636.11
<b>Mean</b>	<b>88.23</b>	<b>21.48</b>	<b>375.46</b>	<b>68.00</b>	<b>354.98</b>	<b>13.68</b>	<b>2.11</b>	<b>621.64</b>
<b>CV, %</b>	<b>6.64</b>	<b>11.27</b>	<b>1.57</b>	<b>13.24</b>	<b>2.04</b>	<b>20.11</b>	<b>33.27</b>	<b>2.47</b>

**Abbreviations:** CP – crude protein; CF – crude fibre; Cfr – crude fat; Ash – crude ash; NFE – nitrogen-free extractable substances; Ca – calcium; P – phosphorus; IVDMD (Aufrère) – in vitro dry matter digestibility determined by the Aufrère method.

Nitrogen-free extractable substances are elements of the chemical composition that affect the taste qualities of grass forage. During the study period, the Felina cultivar had the highest concentration of NFE (360.35 g kg<sup>-1</sup> DM) and P (3.07 g kg<sup>-1</sup> DM) in dry matter. The grasses are also characterized by the lowest content of crude fat (18.43 g kg<sup>-1</sup> DM) and calcium (10.65 g kg<sup>-1</sup> DM) and the lowest in vitro digestibility of dry matter (605.38 g kg<sup>-1</sup> DM). The differences from the average values of the indicators expressed as percentages are as follows: 16.55% (for CF), 28.45% (for Ca), and 2.69% (for IVDMD).

The plant mass of the Lofa cultivar is distinguished by the highest content of CF, minerals, and Ca. The values of the indicators exceed the average by 10.01%, 19.01%, and 26.10%, respectively. The concentration of the macroelement phosphorus is 35.00 to 119.29% lower than that of the other *Festulolium* cultivars included in the study.

The amount of CFr is highest in the self-seeded grasslands of the Hykor cultivar (381.50 g kg<sup>-1</sup> DM). The forage mass of the cultivar also has the lowest mineral content (60.00 g kg<sup>-1</sup> DM). The difference in the values of the indicators compared to the averages is 1.61% and 13.33%, respectively.

The cultivar and age of the grassland have a significant effect on all nitrogen fractions in the dry matter of the studied *Festulolium* cultivars. During the experimental period, the factors year of use/age of the grassland and cultivar had a significant effect  $P < 0.01 - P < 0.001$ ) on the values of the studied traits (Table 3).

T a b l e 3

Degree ( $\eta^2$ ) and significance ( $P$ ) of factorial effects on the main chemical and botanical composition of *Festulolium* cultivars

Indications	Year and age of grassland		Cultivar	
	$\eta^2$ (%)	$P$	$\eta^2$ (%)	$P$
CP	44.97	$P < 0.001$	50.66	$P < 0.001$
CF	4.07	ns	85.21	$P < 0.001$
CFr	1.64	ns	81.58	$P < 0.001$
Ash	51.74	$P < 0.01$	23.63	ns
NFE	3.83	ns	75.99	$P < 0.01$
Ca	18.6	ns	57.24	$P < 0.01$
P	42.83	ns	12.91	ns
IVDMD	13.12	ns	68.54	$P < 0.01$
Botanical composition	89.89	$P < 0.001$	0.66	ns

**Abbreviations:** CP – crude protein; CF – crude fibre; CFr – crude fat; Ash – crude ash; NFE – nitrogen-free extractable substances; Ca – calcium; P – phosphorus; IVDMD – in vitro dry matter digestibility; significance of differences at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$ ; ns – not significant.

The year and age of the grassland ( $\eta^2 = 44.97\%$ ), as well as the cultivar ( $\eta^2 = 50.66\%$ ) significantly influence the CP content in dry matter. Changes in climatic characteristics, as well as the development (age) of grasslands in the growing area, have a significant effect on the mineral ( $\eta^2 = 51.74\%$ ,  $P < 0.01$ ) and botanical ( $\eta^2 = 89.89\%$ ,  $P < 0.001$ ) composition of the formed forage mass. According to the results obtained, the influence of the year/age of the grassland on the content of FC, CF, NFE, Ca, and P is statistically unproven.

Based on the analysis data, the varietal (genetic) trait in *Festulolium* is a significant factor for in vitro dry matter digestibility ( $\eta^2 = 68.54\%$ ,  $P < 0.001$ ) and changes in FC content ( $\eta^2 = 85.21\%$ ,  $P < 0.001$ ) and CF ( $\eta^2 = 81.58\%$ ,  $P < 0.001$ ), which determine the potential energy value of the forage mass. The genotype factor also has a significant effect on the amount of NFE ( $\eta^2 = 75.99\%$ ,  $P < 0.01$ ) and Ca ( $\eta^2 = 57.24\%$ ,  $P < 0.01$ ). The concentration of these indicators is related to the nutritional value of the forage.

The degree and significance of the factorial effects on the basic chemical and botanical composition of *Festulolium* cultivars can optimize variety selection and ecosystem management in mountain regions.

**Conclusions.** The grasslands of the studied *Festulolium* cultivars (Hykor, Lofa, Felina, and Perun) are characterized by low weed infestation, resistance, adaptability, and competitiveness of the species during long-term cultivation in mountain conditions. The crude protein content in the formed forage mass varies from 79.73 to 92.88 g kg<sup>-1</sup> DM, and the in vitro digestibility of dry matter – from 605.38 to 636.11 g kg<sup>-1</sup> DM.

The *Festulolium* hybrids studied are a suitable source for sustainable forage production in the mountainous conditions of the Central Balkan Mountains (Bulgaria).

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