*Naydenova, G., Bozhanska, T., Bancheva, T. (2026). Forage potential of wild-growing populations of Bituminaria bituminosa (L.) Stirt. Agriculture and Forestry, 72 (1): 107-118. https://doi:10.17707/AgricultForest.72.1.07*

DOI: 10.17707/AgricultForest.72.1.07

***Galina NAYDENOVA[1], Tatyana BOZHANSKA*[1], Tsvetomira BANCHEVA[1]***

# FORAGE POTENTIAL OF WILD-GROWING POPULATIONS OF *BITUMINARIA BITUMINOSA* (L.) STIRT.

## SUMMARY

The aim of the present study includes an analysis of the basic chemical composition and an evaluation of the nutritional value of the aboveground biomass from local populations of *Bituminaria bituminosa* (LP 144, LP 191, LP 375, LP 420), harvested at the early flowering phenophase.

On average for the period (2022–2023), the crude protein content in the dry matter of *B. bituminosa* swards was 130.0 g $kg^{-1}$ DM (CV=5.05–11.80%). The analysis data indicate a low degree of variability in the values of this indicator depending on environmental factors. The concentrations of crude fiber, neutral detergent fiber, and acid detergent fiber were 354.4, 483.4, and 375.7 g $kg^{-1}$ DM, respectively. The species recorded high values in terms of the macroelement calcium content (up to 4.79 g $kg^{-1}$ DM) and very low values for phosphorus (0.03–0.08 g $kg^{-1}$ DM).

The values of relative feed value (RFV) in the swards ranged from 95.06 to 138.98%, and the dry matter digestibility (DDM) ranged from 55.18% to 62.75%. These results suggest good intake and assimilation of the forage, especially in dry pasture areas, by ruminant animals.

The analysis data confirm that the high presence of *B. bituminosa* improves the overall forage value of degraded xerothermic grasslands. The species' ability for summer and autumn regrowth makes it a valuable resource for pastures in the hilly and foothill regions of Central Northern Bulgaria.

**Keywords:** *Bituminaria bituminosa*, chemical composition, relative feed value

## INTRODUCTION

Locally adapted legume species are an important component of natural grassland communities. Some of them represent a viable economic alternative for addressing the challenges associated with forage production in areas frequently affected by prolonged droughts (Gil *et al.,* 2016). The identification of new wild

[1]Galina Naydenova, Tatyana Bozhanska (corresponding autor: tbozhanska@mail.bg), Tsvetomira Bancheva, Agricultural Academy, Research Institute of Mountain Stockbreeding and Agriculture, 281 "Vasil Levski" str., Troyan, BULGARIA

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

*Recieved:27/10/2025* *Accepted:16/02/2026*

drought tolerant legume species can extend grazing potential on poorly fertile soils, and areas with low rainfall or prone to drought (Real *et al.*, 2014).

Such a species is tedera (*Bituminaria bituminosa* (L.) Stirt.) which has shown a growing natural distribution in our country (Glogov and Georgieva, 2021). In Northern Bulgaria, it occurs in dry forest-steppe habitats up to 800 m above sea level. The species is drought- and cold-tolerant, adapted to eroded terrains and soils deficient in the macronutrients nitrogen and phosphorus. Niza Costa *et al.* (2023) also identify *B. bituminosa* as the most common early colonizer of burned areas in Spain and, for this reason, use it as a model species for studying the potential of nitrogen-fixing legumes in the restoration of soils and grassland communities after fire events. Our own studies have also reported an increased presence of *B. bituminosa* in post-fire successions in various regions of Bulgaria (Naydenova *et al.*, 2022). In this context, an important question arises: to what extent can this pioneer species contribute forage value to burned natural pastures in the hilly and foothill regions of Central Northern Bulgaria during the early years of their natural recovery? Therefore, the biochemical characterization of naturally occurring ecotypes of *B. bituminosa* in these areas becomes a subject of research interest.

*B. bituminosa* is a perennial legume widely distributed across the Mediterranean Basin and Macaronesia. The plants are grazed by small ruminants but are primarily used as hay for feeding dairy goats, particularly in the Canary Islands (Pazos *et al.*, 2013). According to several international studies, *B. bituminosa* exhibits important ecological advantages as a forage species (Zennouhi *et al.*, 2019; Real *et al.*, 2022; Real, 2022; Fernández-Habas *et al.*, 2022; Fernández-Habas *et al.*, 2025a). In very dry and semi-arid regions with poor soils, it is considered a valuable legume component for grazing by sheep and cattle (Raeside *et al.*, 2013).

Following a comparative assessment of the biochemical and physiological responses to prolonged drought, as well as the recovery capacity after such stress, Foster *et al.* (2015) highlight the advantage of *B. bituminosa* over alfalfa. According to a recent pilot study by Thielecke *et al.* (2024), *B. bituminosa* is among the Mediterranean forage grasses that offer potential for increasing the botanical diversity of grasslands in temperate European countries, particularly during dry and hotter periods in the summer months. Several recent studies on quality parameters identify *B. bituminosa* as a good forage source, demonstrating nutritional value for ruminants comparable to that of major forage crops (Zarat *et al.*, 2020; Bayram *et al.*, 2022; Barbera *et al.*, 2025). According to Barbera *et al.* (2019) the species exhibits high productive and nutritional potential and can be used as an alternative source of natural protein in ruminant diets.

According to our previous studies on semi-natural grasslands in poor and eroded lands in Northern Bulgaria, *B. bituminosa* is the legume with the strongest vegetative growth and the highest summer biomass productivity compared to eight other legume species found in the pasture (Naydenova and Bancheva,

2025). Currently, Bulgarian origins of the species have not been evaluated in terms of quality parameters and, consequently, forage potential.

The aim of the present study is to analyze the basic chemical composition and evaluate the nutritional value of dry forage biomass from local wild populations of *Bituminaria bituminosa*.

## MATERIAL AND METHODS

The experimental work was conducted during the period 2022–2024. In 2022, permanent pastures with a high presence of *B. bituminosa* in the sward were mapped in the hilly and foothill regions of Central Northern Bulgaria. Four populations (LP) from characteristic habitats were selected. The altitudes of the habitats were used as identifiers for the populations in the study, namely: LP 144, LP 191, LP 375 and LP 420. During the following two years (2023–2024), at the early flowering phenophase, mean plant samples were collected from each population through four randomly selected sample plots (1 m2), evenly distributed across the surveyed areas, for biochemical analysis of the aboveground biomass. The analysis included the determination of the basic chemical composition of the dry fodder mass, using the Weende method:

•Crude protein (CP, g $kg^{-1}$ DM) – determined by the Kjeldahl method (according to BDS/ISO-5983);

•Crude fiber (CFr, g $kg^{-1}$ DM);

•Crude fat (CF, g $kg^{-1}$ DM) – determined using Soxhlet extraction (according to BDS/ISO-6492);

•Ash (g $kg^{-1}$ DM) – determined by gradual combustion of the organic matter in a muffle furnace at 550°C (according to BDS/ISO-5984);

•Dry matter (DM, g $kg^{-1}$) – empirically calculated based on moisture content;

•Nitrogen-free extracts (NFE, g $kg^{-1}$ DM) – calculated as: NFE (%) =100– (CP %+CFr %+CF %+Ash %+Moisture %);

•Calcium (Ca, g $kg^{-1}$ DM) – determined by complexometric titration according to Schottz;

•Phosphorus (P, g $kg^{-1}$ DM) – determined with vanadate-molybdate reagent using, measured spectrophotometrically with an Agilent 8453 UV–Visible Spectroscopy System at a wavelength of 425 nm.

Evaluation of feeding value on the basis of fiber components (Linn and Martin, 1991):

•Digestible Dry Matter (DDM, %=88.9 - (0.779 x ADF, %);

•Dry Matter Intake (DMI, %=120/NDF, %);

•Relative Feeding Value (RFV, %=DDM x DMI/1.29).

For the statistical analysis of the obtained data, a two-factor analysis of variance (ANOVA) without replication was performed, with the factors 'habitat/population' and 'year.' Additionally, a multivariate analysis, including principal component analysis (PCA), was conducted, and a biplot model was constructed using singular value decomposition of the first two principal components. All analyses were carried out using the statistical software *Statgraphics* 18 and *Excel* for *Windows* XP.

## RESULTS AND DISCUSSION

### Chemical composition of forage biomass from wild populations of *Bituminaria bituminosa*

At the early flowering phenological stage, the average crude protein (CP) concentration in the dry forage biomass of the studied *B. bituminosa* populations was 130.0 g kg$^{-1}$ DM, ranging from 108.8 in LP 191 to 141.1 g kg$^{-1}$ DM in LP 375 (Table 1).

**Table 1.** Basic chemical composition and structural fiber components of the cell walls in wild populations of *B. bituminosa* (g kg$^{-1}$ DM) – by year and average for the period (2023–2024)

| Populations | CP | CFr | CP / CFr | CF | Ash | NFE | Ca | P | NDF | ADL | Hemi. | Cellul. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| First Year | | | | | | | | | | | | |
| LP 144 | 138.7 | 381.1 | 1/2.7 | 27.9 | 69.6 | 304.4 | 21.2 | 1.4 | 501.1 | 406.4 | 65.7 | 94.7 | 340.7 |
| LP 191 | 108.8 | 386.7 | 1/3.6 | 15.7 | 66.5 | 344.7 | 17.2 | 0.6 | 540.0 | 432.8 | 77.6 | 107.3 | 355.2 |
| LP 375 | 141.1 | 377.7 | 1/2.7 | 22.8 | 68.5 | 310.9 | 21.2 | 0.7 | 500.9 | 406.2 | 89.8 | 94.8 | 316.4 |
| LP 420 | 122.9 | 355.1 | 1/2.9 | 31.4 | 64.7 | 344.9 | 28.0 | 0.6 | 443.6 | 343.1 | 114.8 | 100.5 | 228.3 |
| Mean | 127.9 | 375.2 | 1/3.0 | 24.5 | 67.3 | 326.2 | 21.9 | 0.8 | 496.4 | 397.1 | 87.0 | 99.3 | 310.2 |
| CV | 11.80 | 3.70 | | 27.90 | 3.20 | 6.60 | 20.50 | 46.80 | 8.00 | 9.60 | 24.10 | 6.00 | 18.30 |
| Second Year | | | | | | | | | | | | |
| LP 144 | 125.8 | 338.0 | 1/2.7 | 21.8 | 58.8 | 371.2 | 40.3 | 1.1 | 481.9 | 362.6 | 62.5 | 119.3 | 300.1 |
| LP 191 | 128.3 | 340.9 | 1/2.7 | 13.6 | 61.4 | 373.8 | 34.0 | 0.5 | 500.5 | 345.1 | 69.9 | 155.4 | 275.2 |
| LP 375 | 141.0 | 334.0 | 1/2.4 | 20.0 | 68.0 | 355.6 | 47.0 | 1.0 | 479.4 | 374.0 | 77.4 | 105.4 | 296.6 |
| LP 420 | 132.3 | 321.7 | 1/2.4 | 32.3 | 66.2 | 365.8 | 47.9 | 0.3 | 420.0 | 335.7 | 84.5 | 84.3 | 251.2 |
| Mean | 131.9 | 333.7 | 1/2.5 | 21.9 | 63.6 | 366.6 | 42.3 | 0.7 | 470.5 | 354.4 | 73.6 | 116.1 | 280.8 |
| CV | 5.05 | 2.53 | | 35.39 | 6.67 | 2.20 | 15.34 | 53.27 | 7.42 | 4.85 | 12.90 | 25.75 | 8.04 |
| Average for the period | | | | | | | | | | | | |
| Mean ± Standard error | 129.9 ±3.9 | 354.4 ±8.7 | | 23.2 ±2.4 | 65.5 ±1.3 | 346.4 ±9.3 | 32.1 ±4.3 | 0.8±0.1 | 483.4 ±13.2 | 375.7 ±12.6 | 80.3 ±5.9 | 107.7 ±7.7 | 295.5 ±15.2 |
| *SD* | 11.0 | 24.6 | | 6.9 | 3.7 | 26.3 | 12.1 | 0.4 | 37.3 | 35.7 | 16.7 | 21.9 | 43.0 |
| Factorial effect | | | | | | | | | | | | |
| Year | NS | ** | | NS | NS | * | ** | NS | * | ***P<0.1*** | NS | NS | NS |
| Populations/Habitats | NS | * | | ** | NS | NS | ***P <0.1*** | ***P <0.1*** | ** | NS | ***P <0.1*** | NS | NS |

According to the analysis of variance, differences in climatic conditions between the two experimental years did not significantly affect this key quality parameter. Likewise, variation in the ecological conditions among habitats had no significant effect. The observed crude protein content was significantly lower than that reported for the most widespread local perennial forage species, such as clover and alfalfa (Naydenova *et al.*, 2022; Naydenova and Bozhanska, 2020; Marinova, 2017; Marinova *et al.*, 2024). However, given the high biomass productivity of the studied populations under the strongly limiting conditions of the selected habitats (Naydenova and Ignatova, 2025), the recorded CP values can be considered satisfactory. A synthesis of published data indicates that the protein content of *B. bituminosa* ranges from 159 to 243 g kg$^{-1}$ DM (Gülümser and Acar, 2012). Leaf protein concentration is reported to be nearly twice as high as that of

stems. As small ruminants (sheep and goats) preferentially consume leaf biomass during grazing, the species represents a valuable protein source in degraded xerothermic grasslands dominated by *Chrysopogon gryllus* and in yellow bluestem pastures (*Bothriochloa ischaemum*), where it naturally occurs. Grasslands dominated by *Chrysopogon gryllus* are characterized by limited nutritional value. According to Iliev *et al.* (2017), crude protein content reaches a maximum of 100.4 g $kg^{-1}$ DM even under optimal macromineral fertilization (annual application of $N_6P_6$). The inclusion of *B. bituminosa* in these grassland communities is therefore expected to enhance the overall protein content of the forage mass. This improvement may contribute to higher pasture productivity and increased resilience under drought conditions.

Based on our observations, *B. bituminosa* demonstrates the ability to regrow after seed harvest at the end of July, forming robust leaf rosettes (Figure 1). Thus, the species' presence can significantly increase the yield and quality of semi-natural grasslands when used for grazing in the late summer and autumn periods.

**Figure 1.** Second regrowth of *B. bituminosa* population (LP 375)

The crude fiber (CFr) content in the dry aboveground biomass of *B. bituminosa* is very high, averaging 354.4g $kg^{-1}$ DM. It varies between 321.7 and 386.7g $kg^{-1}$ DM, depending on the yearly ($p<0.01$) and habitat conditions ($p<0.05$). The observed values can be linked to the following biological characteristics of the species: a perennial life cycle and a strong growth habit, a slow developmental rate during the growing season, and flowering under very high summer temperatures, which stimulate the accumulation of structural carbohydrates in the plants. Due to the high fiber content, the crude protein to crude fiber ratio is much lower (ranging from 1:2.4 to 1:3.6) than the optimal ratio of 1:1.2.

Similar to crude fiber, the content of macrominerals and crude fat in the biomass of the species is influenced by environmental factors ($p<0.1$ and $p<0.01$

respectively). For population LP 420, which develops on a steep, heavily eroded slope under the most adverse soil conditions, the highest calcium (Ca) content (47.9 g $kg^{-1}$ DM) and the lowest phosphorus (P) content (0.3 g $kg^{-1}$ DM) were recorded in the second experimental year. The Ca:P ratio in the dry forage mass from this habitat is 160:1, which is many times higher than the critical value of 6:1 (NRC, 2000). The same population also shows significantly higher crude fat content. In a similar study conducted under conditions in Turkey, Bayram *et al.*, (2022) report considerably lower calcium levels (12.3–14.0 g $kg^{-1}$ DM) in the dry biomass of the species and much higher phosphorus contents (3.1–4.1 g $kg^{-1}$ DM). The significant effect of soil and meteorological conditions on the macromineral content of pasture vegetation is well known, with mineral supplementation for animals being essential in dry conditions and regions (Mokolopi, 2019).

The basic forage profile of the species necessitates a comparison between *B. bituminosa* and other naturally occurring legumes in the study region that utilize similar ecological resources. The average contents of crude fat (23.2 g $kg^{-1}$ DM), phosphorus (0.8 g $kg^{-1}$ DM), and ash (65.5 g $kg^{-1}$ DM) in the forage of *B. bituminosa* determined in our study were significantly lower than those recorded for *Medicago falcata* (with corresponding values of 39.7 g $kg^{-1}$ DM, 2.1 g $kg^{-1}$ DM, and 90.8 g $kg^{-1}$ DM), which is the dominant perennial legume species in the dry natural pastures of Central Northern Bulgaria (Naydenova *et al.*, 2022). In contrast, calcium content was higher in *B. bituminosa* –32.1 g $kg^{-1}$ DM compared to 22.4 g $kg^{-1}$ DM in *Medicago falcata*.

The content of neutral detergent fiber (NDF) and acid detergent fiber (ADF) is a key determinant of the relative feed value (RFV) of forage (Kumbasar *et al.*, 2018). The same authors report that ADF and NDF contents in *B. bituminosa* are closely dependent on developmental stage and may range from 198.0 to 471.4 g $kg^{-1}$ DM (ADF) and from 273.7 to 563.8 g $kg^{-1}$ DM (NDF). The average NDF content observed in the biomass of local *B. bituminosa* populations in our study was high –483.4 g $kg^{-1}$ DM, ranging from 420.0 g $kg^{-1}$ DM in LP 420 to 540.0 g $kg^{-1}$ DM in LP 191. Variation across years and habitats was statistically significant ($p<0.05$ and $p<0.01$ respectively).

In both experimental years, population LP 420 recorded the lowest NDF values, which correspond to higher forage quality and greater intake potential by ruminants. This population is located at the highest elevation, on a steep, heavily eroded slope under the most restrictive soil fertility and moisture conditions. In Central Northern Bulgaria, *B. bituminosa* naturally occurs in xerothermic grassland communities dominated by Brachypodium pinnatum or Festuca valesiaca. The studied populations showed significantly lower NDF concentrations compared to the values reported for the dominant grasses in these habitats (610.4 g $kg^{-1}$), as documented by Bozhanska *et al. (*2022). Therefore, the inclusion of *B. bituminosa* in such pasture swards contributes to the improvement of their overall forage quality.

The concentration of acid detergent fiber (ADF) is directly related to forage digestibility and is used in the calculation of total digestible nutrient (TDN) values. The average ADF content determined in our study was 375.7 g $kg^{-1}$ DM. This parameter was not significantly influenced by the ecological conditions of the habitats, although a notable trend was observed toward lower ADF concentrations during the second experimental year (average across all populations: 354.4 g $kg^{-1}$ DM), under conditions of reduced summer precipitation. Based on the observed values, *B. bituminosa* may have significant potential to enhance forage digestibility in the xerothermic pastures where it naturally establishes. This is particularly relevant as the most well-adapted legume species when grazed or cut at early flowering stage, exhibit similar ADF concentrations ranging from 278 to 426 g $kg^{-1}$ DM (Vasiljević *et al.*, 2022).

The biological value of the analyzed biomass also strongly depends on its content of hemicellulose (a fully digestible polysaccharide) and cellulose, which is only partially digestible. According to the results obtained, the hemicellulose content in the dry biomass of the studied local *B. bituminosa* populations was high, justifying the classification of this legume forage as a high-energy feed. The mean values of this parameter were 99.3 g $kg^{-1}$ DM and 116.1 g $kg^{-1}$ DM for the first and second experimental years, respectively. The cellulose fraction in the dry matter was also high, with average values of 310.2 g $kg^{-1}$ DM and 280.8 g $kg^{-1}$ DM, respectively. For both indicators, variation across years and habitats was assessed as insignificant according to the results of the ANOVA.

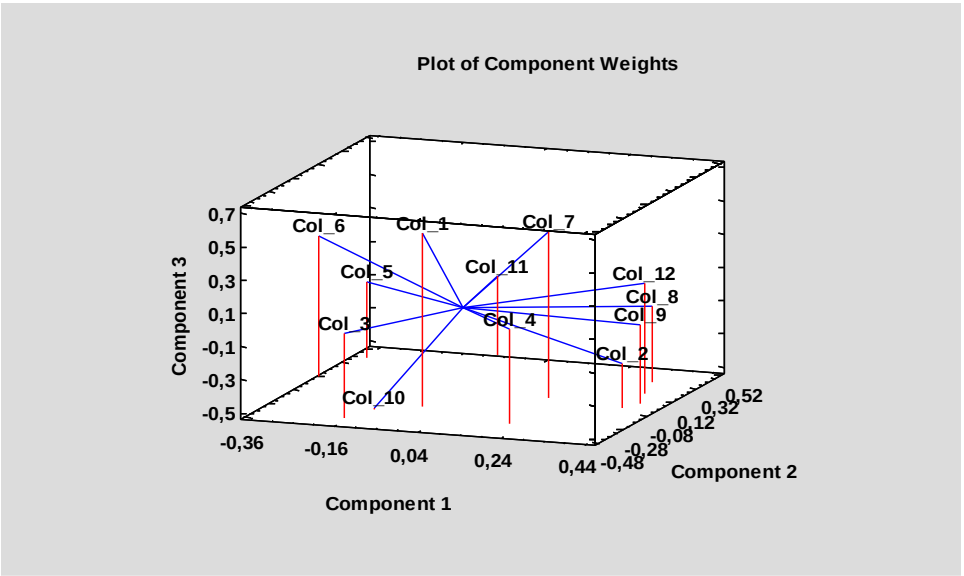
The lignin fraction contributes to the rigidity and structural strength of plant cells but negatively affects forage digestibility (Bonawitz *et al.*, 2010). The quantity of lignified stems in the later stages of *B. bituminosa* development mainly influences the quality of the feed (Fernández-Habas *et al.*, 2025b). At the early flowering stage, the local germplasm of the species recorded relatively low acid detergent lignin (ADL) values, ranging from 62.5 to 114.8 g $kg^{-1}$ DM, with no significant differences between years. A clear trend was observed whereby populations developing at lower elevations and under more favorable soil conditions exhibited lower ADL content.

To assess the variability of the 12 observed indicators of basic chemical composition, as well as the interrelationships among them, a multivariate analysis was conducted (Table 2 and Figure 2).

**Table 2.** Principal components analysis

| Component Number | Eigenvalue | Percent of Variance | Cumulative Percentage |
|---|---|---|---|
| 1 | 5.26272 | 43.856 | 43.856 |
| 2 | 3.24647 | 27.054 | 70.910 |
| 3 | 1.82257 | 15.188 | 86.098 |
| 4 | 0.684814 | 5.707 | 91.805 |
| 5 | 0.659165 | 5.493 | 97.298 |

The analysis was conducted up to the third principal component, which accounted for 86% of the total variation. The variables contributing most significantly to the variation in principal component 1 (PC1), and consequently to the overall variability, were the fiber-related parameters: crude fiber, acid detergent fiber (ADF), neutral detergent fiber (NDF), and cellulose.




*Legend: Col_1 - CP; Col_2 - CFr; Col_3 - CF; Col_4 - Ash; Col_5 - NFE; Col_6 - Ca; Col_7 - P; Col_8 - NDF; Col_9 - ADF; Col_10 - ADL; Col_11 - Hemicell; Col_12 – Cellulose*

**Figure 2. Biplot** Analysis of the Chemical Parameters in Wild Populations of *Bituminaria bituminosa*

These can be considered as the elements of the basic chemical composition most strongly influenced by habitat conditions and harvest years. For the second principal component (PC2), which accounts for 27% of the total variation, the determining factors are the contents of hemicellulose, crude fat, and ash. Protein and macromineral contents are grouped within the same quadrant and have the highest relative loading on principal component 3 (PC3). The key quality indicator (crude protein content) is positively influenced by the levels of the two macrominerals, calcium (Ca) and phosphorus (P), as well as by hemicellulose content.

The assessment of the relative feed value (RFV), which reflects the potential intake and digestibility of the plant biomass (Fekadu *et al.*, 2017), showed low to moderate variability across the studied *B. bituminosa* populations, with RFV values ranging from 95.06% to 138.98% (Table 3). Year-to-year changes in the forage value of *B. bituminosa* revealed relatively low variation in the average values for potential dry matter intake (DMI), which ranged from 2.43% to 2.56%.

**Table 3.** Dry Matter Digestibility and Energy Feed Value of Wild Populations of *Bituminaria bituminosa*

| Populations | DDM | DMI | RFV | GE | EE | FUM | FUG |
|---|---|---|---|---|---|---|---|
| First year | | | | | | | |
| LP 144 | 57.24 | 2.39 | 106.26 | 17.52 | 7.26 | 0.66 | 0.59 |
| LP 191 | 55.18 | 2.22 | 95.06 | 17.15 | 7.21 | 0.66 | 0.59 |
| LP 375 | 57.26 | 2.40 | 106.33 | 17.43 | 7.27 | 0.66 | 0.59 |
| LP 420 | 62.17 | 2.71 | 130.38 | 17.41 | 7.42 | 0.68 | 0.61 |
| ***Mean*** | ***57.96*** | ***2.43*** | ***109.51*** | ***17.38*** | ***7.29*** | ***0.67*** | ***0.60*** |
| ***Sd*** | ***2.97*** | ***0.20*** | ***14.89*** | ***0.16*** | ***0.09*** | ***0.01*** | ***0.01*** |
| Second year | | | | | | | |
| LP 144 | 60.65 | 2.49 | 117.08 | 17.22 | 7.51 | 0.69 | 0.63 |
| LP 191 | 62.02 | 2.40 | 115.26 | 17.08 | 7.47 | 0.69 | 0.63 |
| LP 375 | 59.77 | 2.50 | 115.97 | 17.17 | 7.47 | 0.69 | 0.62 |
| LP 420 | 62.75 | 2.86 | 138.98 | 17.33 | 7.59 | 0.70 | 0.64 |
| ***Mean*** | ***61.30*** | ***2.56*** | ***121.82*** | ***17.20*** | ***7.51*** | ***0.69*** | ***0.63*** |
| ***Sd*** | ***1.34*** | ***0.20*** | ***11.46*** | ***0.10*** | ***0.06*** | ***0.01*** | ***0.01*** |

*Legend: DDM (%); DMI (%); RFV-relative (%); GE (MJ/kg DM); EE (MJ/kg DM); FUM (n in kg DM); FUG (n in kg DM)*

The energy feed value of forage encompasses both the biological metabolism and the quantitative ratio of nutrients in the dry matter. It is a key criterion in the modern evaluation of biomass quality and is typically expressed through feed units for milk and growth (Todorov, 2010). The gross energy (GE) and energy required to support the basic physiological functions in animals (EE) in the vegetative biomass of *B. bituminosa* correlate with the fiber components of the cell walls, which represent the primary source of nutritional energy for ruminants.

In the present study, the variability in these parameters was low, with GE values ranging from 17.08 MJ/kg DM (LP 191) to 17.52 MJ/kg DM (LP 144), and EE ranging from 7.21 MJ/kg DM (LP 191) to 7.59 MJ/kg DM (LP 420). This trend also persisted in the values of net energy (FUM and FUG). On average over the study period, the number of feed units in the wild populations of *B. bituminosa* ranged from 0.66 to 0.70 per kg of dry matter (FUM), and from 0.59 to 0.64 per kg of dry matter (FUG).

The dry matter digestibility (DDM) of the studied *B. bituminosa* populations was relatively high. The empirically calculated DDM values showed low variability as well, ranging from 55.18% (LP 191) to 62.75% (LP 420).

## CONCLUSIONS

Across four habitats located at altitudes between 144 and 420 m, the local germplasm of *B. bituminosa* was evaluated over two growing seasons. The habitats differed in edaphic and climatic conditions. On average, the dry aerial biomass harvested at the beginning of the flowering stage showed the following basic chemical composition: crude protein (CP)–130.0 g $kg^{-1}$ DM; crude fiber (CFr)–354.4 g $kg^{-1}$ DM; neutral detergent fiber (NDF)–483.4 g $kg^{-1}$ DM; acid detergent fiber (ADF)–375.7 g $kg^{-1}$ DM; hemicellulose–107.7 g $kg^{-1}$ DM; cellulose– 295.5 g $kg^{-1}$ DM; acid detergent lignin (ADL)–88.6 g $kg^{-1}$ DM; crude

fat (CF)–23.2 g $kg^{-1}$ DM; ash–65.5 g $kg^{-1}$ DM; calcium (Ca)–32.1 g $kg^{-1}$ DM; and phosphorus (P)–0.8 g $kg^{-1}$ DM. The contents of crude fiber, macrominerals, and crude fat were significantly influenced by ecological variability. The species showed very high hemicellulose and calcium concentrations. In contrast, phosphorus content was extremely low. Fiber-related parameters were the main drivers of variability in chemical composition. These included crude fiber, neutral and acid detergent fiber, and cellulose. They formed the first principal component in the multivariate analysis. This indicates a strong dependence on habitat characteristics, particularly soil properties, altitude, and harvest year. The relative feed value of the biomass ranged from 95.06 to 138.98%. This indicates that the inclusion of *B. bituminosa* in xerothermic pastures positively affects biomass quality.

Given the proven feed value of the species under limiting environmental conditions, further research should focus on evaluating the potential for its integration into semi-natural grasslands, for example through direct overseeding following fire events. The investigated genetic material may also serve as a valuable initial resource for breeding programs, as it exhibits the key agronomic trait of specific adaptation to local regions, environmental conditions, and targeted production systems.

## ACKNOWLEDGEMENTS

This research was supported by the Bulgarian National Science Fund (BNSF), under Project No. KP-06-H86/1, dated 05.12.2024, titled "Metagenomic, botanical and soil studies of burned meadows and pastures as a basis for their restoration".

## REFERENCES

Barbera, M., Mendoza-Grimón, V., Espinosa, J., Rodriguez-Ponce, E., Gracia, A., SanJuán, E., & Ventura, M. R. (2025). Feed intake, preferences, in vivo digestibility, and nutritional value of tedera (*B. bituminosa* var. *bituminosa*) and maralfalfa (*Pennisetum purpureum*) in Canary sheep. Tropical Animal Health and Production, 57(2): 1-10. https://doi.org/10.1007/s11250-025-04284-z

Barbera, M., Muñoz, M., Rodriguez-Ponce, E., Ventura, M. E. (2019). Potential value of tedera (*B. bituminosa*) as high protein resource for poultry feed. Trop Anim Health Prod., 51: 465-468 https://doi.org/10.1007/s11250-018-1677-5

Bayram, G. K., Gülümser, E., Mehmet, C. A. N., Zeki, A. C. A. R. & İlknur, A. Y. A. N. (2022). *B. bituminosa* (L.) CH Stirt. genotiplerinin farklı gelişme dönemlerinde kaba yem kalitesi. ISPEC Journal of Agricultural Sciences, 6(3): 511-519.

Bonawitz, N. D., Chapple, C., Campbell, A., Lichten, M. & Schupbach, G. (2010). The genetics of lignin biosynthesis: connecting genotype to phenotype. Annual Review of Genetics, 44: 337-363.

Bozhanska, T., Iliev, M., Bozhanski, B. & Petkova, M. (2022). Impact of fertilizing with organic composted mixture on the quality of natural grass stands in the semi-mountain regions of Bulgaria. Ecologia Balkanica, 14(2): 39-50. http://web.uni-plovdiv.bg/mollov/EB/2022_vol14_iss2/039-050_eb22133.pdf

Fekadu, D., Walelegn, M. & Terefe, G. (2017). Indexing Ethiopian Feed Stuffs Using Relative Feed Value: Dry Forages and Roughages, Energy Supplements, and Protein Supplements. Journal of Biology, Agriculture and Healthcare, 7(21): 57-60.

Fernández-Habas, J., Real, D., Vanwalleghem, T., Leal-Murillo, J. R. & Fernández-Rebollo, P. (2025b). Yield and Forage Quality of the New Forage Perennial Legume *B. bituminosa* var. albomarginata cv. Lanza in Response to Rainfall Reduction and Competition. Journal of Agronomy and Crop Science, 211(1): e70013. https://doi.org/10.1111/jac.70013

Fernández-Habas, J., Real, D., Vanwalleghem, T., Leal-Murillo, J. R. & Fernández-Rebollo, P. (2025a). Effect of Rainfall Reduction and Competition on the Phenology of the Mediterranean Forage Perennial Legume *B. bituminosa* var. albomarginata cv. Lanza. Journal of Agronomy and Crop Science, 211(4): e70104. DOI: 10.1111/jac.70104

Fernández-Habas, J., T. Hidalgo-Fernández, M., Leal-Murillo, J. R., Méndez, P., Quero, J. L., Vanwalleghem, T. & Fernández-Rebollo, P. (2022). Effects of two water regimes on morphological traits, nutritive value and physiology of three *B. bituminosa* varieties from the Canary Islands. Journal of Agronomy and Crop Science, 208(4): 413-426. https://doi.org/10.1111/jac.12485

Foster, K., Lambers, H., Real, D., Ramankutty, P., Cawthray, G. R. & Ryan, M. H. (2015). Drought resistance and recovery in mature *B. bituminosa* var. albomarginata. Annals of Applied Biology, 166(1): 154-169.

Gil, V., Rey, L., Barberá, M., Castanon, I. R. & Ventura, M. R. (2016). Tannin content and chemical composition of unconventional and conventional feed for ruminants. Agriculture & Forestry, 62(4): 05-10, Podgorica. DOI: 10.17707/AgricultForest.62.4.01

Gülümser, E. & Acar, Z. (2012). Morphological and chemical characters of *Bituminaria obituminosa* (L) CH (Stirtion) grown naturally in Middle Black Sea Region. Turkish Journal of Field Crops, 17(2): 101-104.

Iliev, M., Mihovski, T. & Churkova, B. (2017). Influence of Biostim organic fertilizer on the bioproductive indicators of forage of natural grassland of the type *Chrysopogon grullus* L. in the region of the Central Balkan Mountain. Journal of Mountain Agriculture on the Balkans, 20(5): 136-14.

Kumbasar, F., Acar, Z., Gulumser, E., Can, M. & Ayan, I. (2018). Determination of morphological, agricultural and quality parameters at different growth stage of *B. bituminosa* genotypes. Fresenius Environmental Bulletin, 27(7): 5078-5084.

Linn, J. C. & Martin, N. P. (1991). Forage quality analysis and interpretation, Vet. Clin. North Am.: Food Anim. Practice, 7(2): 509-523.

Marinova, D. (2017). Variability and relationships of some important alfalfa germplasm traits. Banat's Journal of Biotechnology, 8(15): 18-24.

Marinova, D., Dyakova, G. & Mincheva, R. (2024). Breeding evaluation of alfalfa (*Medicago sativa* L.) polycross progenies by multivariate analysis based on agro-morphological traits. Comptes rendus de l' Académie bulgare des Sciences (Proceedings of the Bulgarian Academy of Sciences), 77(12): 1870-1878.

Mokolopi, B. G. (2019). Phosphorus, calcium, and magnesium contents of pasture and their effect on body condition scores and body mass of communal cattle depending on natural pasture of Mogosane Village, of the North-West Province, South Africa. Tropical Animal Health and Production, 51(7): 2067-2071.

Naydenova, G. & Bozhanska, T. (2020). The biodiversity, the relative share and some forage quality traits of clover species (Trifolium spp.) in the grasslands of the Central Northern Bulgaria. Analele Universităţii din Oradea, Fascicula Biologie, 27(2): 116-122. https://www.bioresearch.ro/2020-2/116-122-AUOFB.27.2.2020-NAYDENOVA.G.-The.biodiversity.pdf

Naydenova, G. & Ignatova, A. (2025). Functional characteristics of local wild-growing germplasm from tedera (*B. bituminosa* (L.) Stirt.). Bulgarian Journal of Soil Science Agrochemistry and Ecology, 59(3): 36-48.

Naydenova, G., & Bancheva, T. (2025). Species and Functional Diversity of the Legume Component in Yellow Bluestem Pastures (Type *Bothriochloa ischaemum*). Proceedings of the Bulgarian Academy of Sciences 78(12): 1895-1902.

Naydenova, G., Bozhanski, B. & Bozhanska, T. (2022). Wild alfalfa in the semi-natural grasslands of Central Northern Bulgaria. Scientific Papers. Series A. Agronomy, LXV(1): 447-454. https://agronomyjournal.usamv.ro/pdf/2022/issue_1/Art65.pdf

Raeside, M. C., Nie, Z. N., Clark, S. G., Partington, D. L., Behrendt, R. & Real, D. (2013). Evaluation of tedera [(*B. bituminosa* (L.) CH Stirton var. albomarginata)] as a forage alternative for sheep in temperate southern Australia. Crop and Pasture Science, 63(12): 1135-1144.

Real, D. (2022). Critical Agronomic Practices for Establishing the Recently Domesticated Perennial Herbaceous Forage Legume Tedera in Mediterranean-Like Climatic Regions in Western Australia. Agronomy, 12(2): 274. https://doi.org/10.3390/agronomy12020274.

Real, D., Bennett, R. G., Nazeri, N. K. & Weaver, D. M. (2022). Critical P, K and S Concentrations in Soil and Shoot Samples for Optimal Tedera Productivity and Nodulation. Agronomy, 12(7): 1-20. https://doi.org/10.3390/agronomy12071581.

Real, D., Oldham, C. M., Nelson, M. N., Croser, J., Castello, M., Verbyla, A., Pradhan, A., Van Burgel, A., Méndez, P., Correal, E., Teakle, N. L., Revell, C. K. & Ewing M. A. (2014). Evaluation and breeding of tedera for Mediterranean climates in southern Australia. Crop and Pasture Science, 65(11): 1114-1131. https://doi.org/10.1071/CP13313

Thielecke, D., Isselstein, J. & Komainda, M. (2024). Multispecies for multifunctional grassland: Evaluation of minor and novel forage species for temperate humid grasslands. Annals of Applied Biology, 185(1): 91-107.

Todorov, N. (2010). Practice in Animal Nutrition. East-West, Sofia, Bulgaria, pp. 463.

Vasiljević, S., Radinović, I., Branković, G., Krstić, S., Prodanović, Sl., Živanović, T. & Katanski, S. (2022). Evaluation of a diverse collection of red clover for forage quality and antioxidant activity. Biotechnol. Agron. Soc. Environ., 2022 26(4): 210-223. https://popups.uliege.be/1780-4507/index.php?id=19967&file=1

Zarat, L., Alfaiz, C., Chiadmi, N., Kallida, R., Gaboun, F. & Ibriz, M. (2020). Plant growth, physiological response and osmotic adjustment of wild *B. bituminosa* (L.) stirton accessions under salt stress. Plant Cell Biotechnology and Molecular Biology, 21(1&2):59-69.

Zennouhi, O., Mderssa, M. El., Ibijbijen, J., Bouiamrine, E. & Nassiri, L. (2019). The Use of *B. bituminosa* (L.) Stirton and Microbial Biotechnologies for Restoration of Degraded Pastoral Lands: The Case of the Middle Atlas of Morocco. International Journal of Research in Environmental Science 5(2): 1-6. DOI: http://dx.doi.org/10.20431/2454-9444.0502001