

## EVALUATION OF THE FORAGE QUALITY OF SOME MIXTURES OF AROMATIC MEDICINAL PLANTS AND GRASSLAND GRASS SPECIES WITH A VIEW TO OPTIMIZING THE NUTRITIONAL VALUE

Sorina NIȚU (NĂSTASE)<sup>1,2</sup>, Andreea ANDREOIU<sup>1</sup>, Monica TOD<sup>1</sup>

<sup>1</sup>Research and Development Grasslands Institute, 5 Cucului Street, 500128, Braşov, Romania

<sup>2</sup>University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca,  
3-5 Calea Manastur, 400372, Cluj-Napoca, Romania

Corresponding author email: andreea.andreoiu@pajisti-grassland.ro

### Abstract

*Cultivated grasslands are composed of a diverse mix of plant species with different forage qualities. This study aimed to evaluate the quality of forage resulted from grassland grass species and aromatic medicinal plants, in order to optimize their nutritional value and forage potential. The study was conducted at ICDP Braşov, Romania, within a field experiment of 5 variants of plant mixtures with 20 species of aromatic medicinal plants and grasses. The main quality parameters that were analyzed were: crude protein (CP), total ash (ASH), crude fiber (CF), acid detergent fiber (ADF), lignin (ADL), neutral detergent fiber (NDF), digestibility dry matter (DMD) and digestibility organic matter (DOM). The highest CP content was obtained in variants V3 (20.33%) and V5 (19.50%), compared to the control variant V1 (17.40%). The highest ADF content was recorded in the control variant V1 (34.57%), compared to the other 4 variants, which recorded lower values, with positive implications on the quality of the feed. The correlations between the analyzed parameters showed that crude protein was positively correlated with total ash, dry matter digestibility and organic matter.*

**Key words:** aromatic medicinal plants, fodder quality, grasses, grassland.

### INTRODUCTION

Grasslands are the main agricultural land use both at European and global scale (Stypinski, 2011; Török et al., 2021). Forage crops have served as a source of feed of domestic animals and cultivated to produce high yields of feed/fodder, which are also rich in nutrients suitable for animal's requirements (Kumar et al., 2018). The growing of medicinal and aromatic plants can improve the cropping systems, and ultimately improve the fertility status of field. Integrating these crops into livestock diets, alongside other fodder sources, will enhance both performance and health status while protecting animals from major diseases (Kirti and Arya, 2019).

Medicinal plants remain a primary, and sometimes the sole, therapeutic source and a viable option for livestock management. Their use is driven by lower production costs and the ability to replace antibiotics, thereby preventing antibiotic residues in food products. Furthermore, this approach mitigates problems associated with antibiotic-resistant bacteria and,

consequently, enhances public health by improving food safety (Salmerón - Manzano et al., 2020; Oluwafemi et al., 2020).

Sustainable Intercropping forage-medicinal plants plays an important role in farming systems that can improve the light absorption and soil conservation compared to monoculture (Huss et al., 2022). Plant functional types and traits may also influence forage nutritive values (Gardarin et al., 2014; Blaix et al., 2023; Tasset et al., 2019). This study aimed to evaluate the quality of forage resulted from grassland grass species mixtures of aromatic medicinal plants and, in order to optimize their nutritional value.

### MATERIALS AND METHODS

The research was established and monitored within the Institute for Research and Development for Grasslands (ICDP) from Braşov, Romania, within the Laboratory of Medicinal and Aromatic Plants.

The experimental field of the ICDP Braşov is located in the Braşov Depression located at 25°45' East longitude and 45°42' North latitude,

and the altitude at which the experimental field is located is 520 m.

The climate of the Braşov Depression falls into the temperate-continental type, representing a transition zone between the influences of the temperate oceanic climate and those of the temperate continental climat.

The present study provides partial results of a three-year internal research initiative (2024-2027) conducted at the Research and Development Institute for Grassland Braşov (ICDP Braşov). This research focuses on improving the nutritional quality of forages from sown grasslands through the use of optimized seed mixtures. The central working hypothesis

aims to increase the floristic diversity of sown phytocenoses by incorporating medicinal and aromatic plant taxa, thereby developing more resilient and functional feed resources for livestock. The research focused on the study of five variants (V1 to V5) of mixtures of grasses and some medicinal plant species. The basic structure, common to all variants, consisted of a mixture of perennial grasses: *Lolium perenne* (9 g), *Dactylis glomerata* (6 g) and *Festuca arundinacea* (5 g), to which variable proportions of medicinal and aromatic plants were added, depending on the Thousand Grain Weight (TGW) and the plant habit are presented in Table 1.

Table 1. Composition of experimental variants

| Species                                       | Botanical family | V1   | V2     | V3   | V4    | V5   |
|-----------------------------------------------|------------------|------|--------|------|-------|------|
| <i>Lolium perenne</i>                         | Poaceae          | 9 g  | 9 g    | 9 g  | 9 g   | 9 g  |
| <i>Dactylis glomerata</i>                     | Poaceae          | 6 g  | 6 g    | 6 g  | 6 g   | 6 g  |
| <i>Festuca arundinacea</i>                    | Poaceae          | 5 g  | 5 g    | 5 g  | 5 g   | 5 g  |
| <i>Ocimum basilicum</i>                       | Lamiaceae        | -    | 2 g    | -    | -     | 1 g  |
| <i>Dracocephalum moldavica</i>                | Lamiaceae        | -    | 1.5 g  | -    | -     | -    |
| <i>Agastache foeniculum</i>                   | Lamiaceae        | -    | -      | 1 g  | -     | -    |
| <i>Sideritis scardica</i>                     | Lamiaceae        | -    | -      | 2 g  | -     | -    |
| <i>Satureja hortensis</i>                     | Lamiaceae        | -    | -      | -    | 1 g   | -    |
| <i>Salvia sclarea</i>                         | Lamiaceae        | -    | -      | -    | 1 g   | -    |
| <i>Hyssopus officinalis</i>                   | Lamiaceae        | -    | -      | -    | 1 g   | -    |
| <i>Monarda citriodora</i>                     | Lamiaceae        | -    | -      | -    | -     | 1 g  |
| <i>Calendula officinalis</i>                  | Asteraceae       | -    | -      | 5 g  | -     | -    |
| <i>Echinacea purpurea</i>                     | Asteraceae       | -    | -      | 2 g  | -     | -    |
| <i>Leucanthemum vulgare</i>                   | Asteraceae       | -    | -      | 1 g  | -     | -    |
| <i>Cichorium intybus</i>                      | Asteraceae       | -    | -      | -    | -     | 1 g  |
| <i>Trigonella foenum-graecum</i>              | Fabaceae         | -    | -      | -    | -     | 2 g  |
| <i>Cicer arietinum</i>                        | Fabaceae         | -    | 50b*   | -    | -     | -    |
| <i>Lupinus albus</i>                          | Fabaceae         | -    | -      | -    | 100b* | -    |
| <i>Phacelia tanacetifolia</i>                 | Boraginaceae     | -    | 4 g    | -    | -     | -    |
| <i>Nigella damascena</i>                      | Ranunculaceae    | -    | 2 g    | -    | -     | -    |
| <i>Linaria vulgaris</i>                       | Plantaginaceae   | -    | -      | -    | 2 g   | -    |
| <i>Hypericum perforatum</i>                   | Hypericaceae     | -    | -      | -    | -     | 1 g  |
| Total seed quantity (g)/6 m <sup>2</sup> plot |                  | 20 g | 29.5 g | 31 g | 25 g  | 26 g |

b\* = seed count; these quantities are excluded from the total gravimetric calculation

The percentage distribution of botanical families (Poaceae, Lamiaceae, Asteraceae, Boraginaceae, Ranunculaceae, Plantaginaceae,

Fabaceae and Hypericaceae) for the five experimental variants is presented in Figure 1.

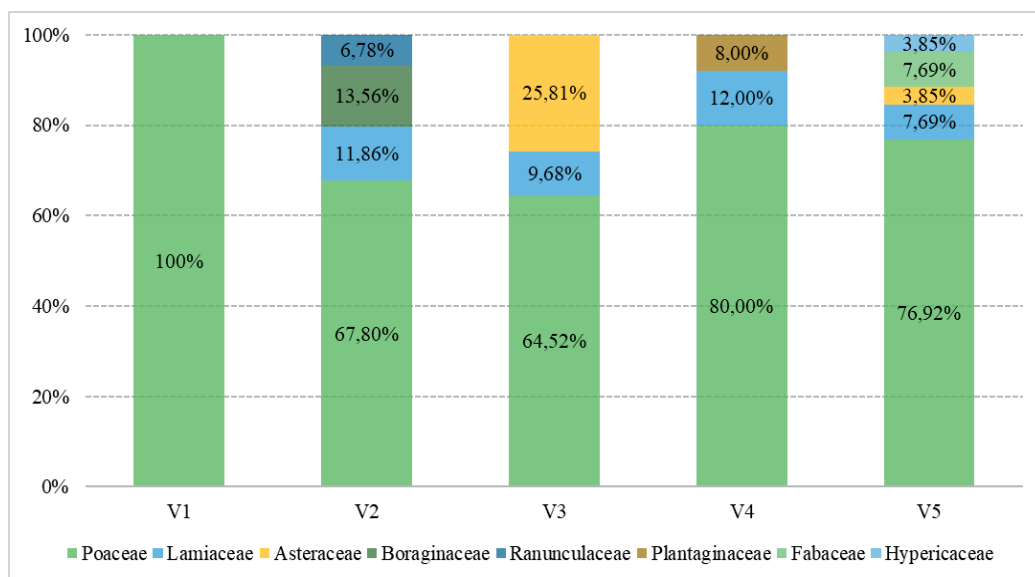


Figure 1. Distribution of botanical families across experimental variants

The experiment was designed on a single-factor model in triplicates, using the randomized block design. The plots were sized at 6 m<sup>2</sup>, with a length of 4 m, and a width of 1.5 m. A 25 cm protection zones was left between variants, and 2 m wide alleys were drawn between the blocks (repeats).

To determine the qualitative parameters, plant mass sampling was conducted chronologically for each experimental variant. Specifically, one representative sample was collected from each of the three biological replicates, resulting in a total of three samples per variant. This procedure ensured statistical consistency and scientific rigor across all experimental plots. The samples were dried at 60°C and then ground with a Grindomix GM 200 mill and prepared for the chemical parameter's analysis.

The chemical parameters analysis was carried out by the Near Infrared Spectroscopy (NIR) method with Perten DA 7200, as follows: crude protein (CP), ash (ASH), crude fiber (CF), acid detergent fiber (ADF), lignin (ADL), neutral fiber detergent (NDF), digestibility of the dry matter (DMD) and the digestibility of the organic matter (DOM). Forage quality was evaluated using two standardized forage grading systems: Relative Feed Value (RFV) and Relative Feed Quality (RFQ). These systems provide a numerical index to rank cool-season legumes, grasses, and mixtures. While the RFV

system (Rohweder et al., 1978) is based on potential digestible dry matter intake calculated from ADF and NDF, the RFQ system (Moore and Undersander, 2002) is a more recent evaluation system that incorporates in vitro fiber digestibility to provide a more accurate prediction of animal performance. All the data was statistically analysed using ANOVA and DUNCAN Test.

## RESULTS AND DISCUSSIONS

The results obtained from the analyses performed on the feed samples and the statistical calculations regarding the content of crude protein, crude ash, crude fiber and acid detergent fiber, are presented in Table 2. The variants with the highest average crude CP are V3 (20.33%) and V5 (19.50%), both of which were statistically significantly different from V1, V2 and V4 according to Duncan's test. The increase of CP in variants V3 and V5 can be attributed to the inclusion of nitrogen-fixing species (legumes) and other species, such as *Cichorium intybus* L., with high protein potential, and high forage quality (Iqbal et al., 2022; Nwafor et al., 2017). In terms of forage quality, grasses are often characterized by lower protein levels than those found in leguminous and forb species (Lee, 2018).

Crude ash, which represents the mineral content remaining after calcination, ranged from 9.80%, in V2, to 10.60%, in V3. But, highest average values for ASH were registered in V3 (10.60%) and V5 (10.57%) variants, indicating a significantly higher total mineral content compared to V1 and V2 variants. This suggests an efficient accumulation of minerals from the soil by the diverse species included in the mixtures, possibly due to the varied root systems and intrinsic absorption capacity of plants such as *Cichorium intybus* (adapted to calcareous soils) (Song et al., 2025). Regarding the crude fiber content, the highest value, with negative indications, was obtained in V1 (30.77%), the variant with only grass

species, being more fibrous. The lowest values of CF were obtained in V2 (27.17%), V3 (26.10%) and V5 (26.83%) variants. The presence of aromatic and medicinal plants in V2, V3, V4 and V5 contributes to the reduction of fiber content, compared to V1. A similar pattern was observed for the fiber fractions. Specifically, variant V1 recorded the highest acid detergent fiber (ADF) content (34.57%), being significantly different from variants V2, V3, and V5. In contrast, variants V2, V3 (both 30.70%), and V5 (30.83%) exhibited the lowest ADF values. These lower concentrations indicate a higher energy potential and superior digestibility for these specific variants.

Table 2. Evaluation of CP, ASH, CF and ADF in feed samples

|                   | Variant        | CP (%)   | ASH (%) | CF (%)   | ADF (%)  |
|-------------------|----------------|----------|---------|----------|----------|
| V1                | Mean           | 17.40 a  | 9.90 a  | 30.77 b  | 34.57 b  |
|                   | Std. Deviation | 0.9644   | 0.1732  | 17.786   | 15.567   |
|                   | Variance       | 0.930    | 0.030   | 3.163    | 2.423    |
| V2                | Mean           | 17.73 a  | 9.80 a  | 27.17 a  | 30.70 a  |
|                   | Std. Deviation | 17.926   | 0.1732  | 32.005   | 32.512   |
|                   | Variance       | 3.213    | 0.030   | 10.243   | 10.570   |
| V3                | Mean           | 20.33 b  | 10.60 b | 26.10 a  | 30.70 a  |
|                   | Std. Deviation | 13.317   | 0.3000  | 17.000   | 10.149   |
|                   | Variance       | 1.773    | 0.090   | 2.890    | 1.030    |
| V4                | Mean           | 17.77 a  | 10.17b  | 29.10 ab | 33.10 ab |
|                   | Std. Deviation | 12.014   | 0.3215  | 27.622   | 31.480   |
|                   | Variance       | 1.443    | 0.103   | 7.630    | 9.910    |
| V5                | Mean           | 19.50 ab | 10.57 b | 26.83 a  | 30.83 a  |
|                   | Std. Deviation | 0.3000   | 0.2517  | 10.504   | 11.719   |
|                   | Variance       | 0.090    | 0.063   | 1.103    | 1.373    |
| Duncan test DL 5% |                | 2.00     | 0.51    | 3.39     | 3.51     |

The results regarding lignin content, neutral detergent fiber, dry matter digestibility and organic matter digestibility are presented in Table 3. The lowest value of ADL was obtained in variant V5 (2.47%), and the highest value was obtained in variant V4 (3.23%). In the variants V1, V2 and V3 was obtained the same lignin content (2.70%). Regarding the NDF content, the lowest (ideal) value was recorded in variants V2, V3 and V5 (approximately 55-55.5%). These had a significantly lower total structural fiber content, which allows for higher feed intake and more efficient digestion, compared to variant V1, which had the highest value (62.43%) of NDF.

The dry matter digestibility coefficients had the highest value in variant V5 (74.17%). The complex mixture that includes *Monarda citriodora* and *Cichorium intybus*, seems to have generated the best nutritional balance. The highest values of NDF were obtained in V1 (62.43%) and V4 (58.27%) variants, the mixtures being more difficult to digest, either due to the higher accumulation of lignin in variant V4, or the excess of fiber in variant V1. The highest value of organic matter digestibility was obtained in variant V5 (69.13%), ranked first (letter "b" in the Duncan test) indicating a clear superiority. The mixture of medicinal herbs and chicory offers a much "easier" feed digestion.

Table 3. Evaluation of ADL, NDF, DMD and DOM in feed samples

|                     | Variant        | ADL (%) | NDF (%)  | DMD (%) | DOM (%)  |
|---------------------|----------------|---------|----------|---------|----------|
| V1                  | Mean           | 2.70 ab | 62.43 b  | 67.57 a | 63.03 ab |
|                     | Std. Deviation | 0.1000  | 26.006   | 26.407  | 20.133   |
|                     | Variance       | 0.010   | 6.763    | 6.973   | 4.053    |
| V2                  | Mean           | 2.70 ab | 55.37 a  | 71.17 a | 66.03 ab |
|                     | Std. Deviation | 0.3606  | 51.248   | 61.175  | 56.323   |
|                     | Variance       | 0.130   | 26.263   | 37.423  | 31.723   |
| V3                  | Mean           | 2.70 ab | 55.20 a  | 71.40 a | 66.13 ab |
|                     | Std. Deviation | 0.4583  | 0.3000   | 25.239  | 31.005   |
|                     | Variance       | 0.210   | 0.090    | 6.370   | 9.613    |
| V4                  | Mean           | 3.23 b  | 58.27 ab | 67.40 a | 62.70 a  |
|                     | Std. Deviation | 0.3055  | 61.093   | 45.530  | 40.731   |
|                     | Variance       | 0.093   | 37.323   | 20.730  | 16.590   |
| V5                  | Mean           | 2.47 a  | 55.53 a  | 74.17 a | 69.13 b  |
|                     | Std. Deviation | 0.2517  | 32.347   | 15.011  | 15.885   |
|                     | Variance       | 0.063   | 10.463   | 2.253   | 2.523    |
| Duncan test (DL 5%) |                | 0,50    | 6,57     | 6,34    | 5,89     |

To provide a comprehensive assessment of the forage quality beyond individual chemical components, the Relative Feed Value (RFV) and Relative Feed Quality (RFQ) were calculated. These integrated indices allow for a standardized ranking of the experimental variants, reflecting their potential for animal intake and energy availability. The performance of the experimental variants regarding these quality indicators is illustrated in Figure 2.

The RFV values ranged from 92.49 to 110.52. Based on the quality scales established by Undersander and Moore (2002) and Jeranyama (2024), variants V2, V3, and V5 are classified as having “fair quality” (103-124), whereas V1 and

V4 fall into the “low quality” category (below 102).

A more dynamic pattern was observed for the RFQ index, with values fluctuating between 99.61 and 117.88. Notably, variant V2 achieved the highest RFQ score (117.88). This superior performance confirms the high nutritional value of this specific mixture, likely due to the inclusion of *Cicer arietinum*. The presence of this legume enhances the overall digestibility and protein profile of the forage, leading to a more favorable RFQ compared to the grass-dominant variant V1, which recorded the lowest value (99.61)

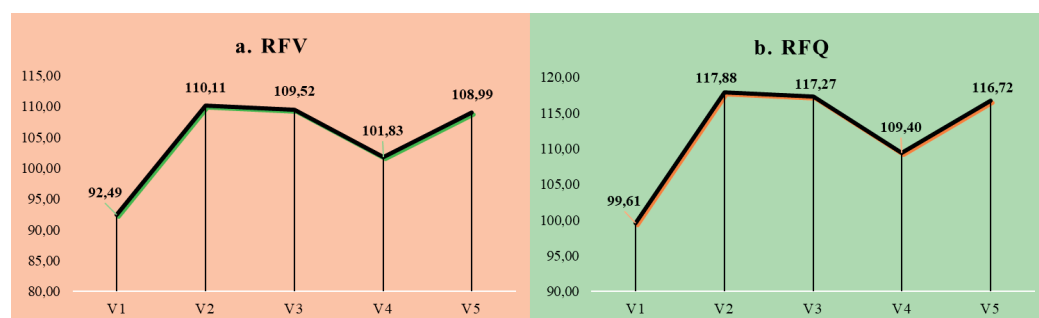


Figure 2. Index variation Relative Feed Value (RFV) and Relative Forage Quality (RFQ) for variants

Correlation analysis is an important source for understanding the degree of association between two or more variables (Andreoiu and Gruia, 2023, Chandra et al., 2012), providing critical insights into the internal dynamics of forage

quality. In the context of forage science, identifying strong associations between protein and digestibility is a standard approach to predict animal performance (Van Soest, 1994).

The Pearson correlation coefficients obtained in this study are detailed in Table 4.

There was a statistically significant positive correlation ( $p \leq 0.01$ ) between crude protein content (CP) and crude ash (ASH), dry matter digestibility (DMD) and digestibility organic matter (DOM) (0.812\*\* and 0.808\*\*), suggesting that feeds from mixtures with more crude protein tend to have better overall digestibility. On the other hand, the correlations between crude protein (CP) and crude fibre (CF), acid detergent fiber (ADF), neutral detergent fiber

(NDF) were negative ( $p \leq 0.01$ ). There was a very strong positive correlation (0.980\*\*) between ADF and NDF, which is logical since ADF is a sub-fraction of NDF (Saha et al., 2010). Strong negative correlations (between -0.61\* and -0.92\*\*) existed between ADF, NDF and ADL and digestibility dry matter (DMD) and organic matter (DOM), confirming that higher fiber and lignin content significantly reduces the digestibility of the feed by the animal (Moore and Jung 2001).

Table 4. Correlations between the main indicators analyzed

| Characters | CP        | ASH     | CF       | ADF      | ADL      | NDF      | DMD    |
|------------|-----------|---------|----------|----------|----------|----------|--------|
| ASH        | 0.862**   |         |          |          |          |          |        |
| CF         | -0.857**  | -0.610* |          |          |          |          |        |
| ADF        | -0.0780** | -0.525* | 0.980**  |          |          |          |        |
| ADL        | -.0555*   | -0.409  | 0.441    | 0.422    |          |          |        |
| NDF        | -0.705**  | -0.500  | 0.928**  | 0.969**  | 0.224    |          |        |
| DMD        | 0.812**   | 0.609*  | -0.899** | -0.921** | -0.663** | -0.847** |        |
| DOM        | 0.808**   | 0.615*  | -0.878** | -0.889** | -0.716** | -0.792** | 0.99** |

Significance levels: \* $P < 0.05$ ; \*\* $P < 0.01$ .

CONCLUSIONS

Overall, diversifying the floristic composition within our experiment by including different species of aromatic/medicinal plants species, as well as other species from different botanical families in the mixtures made for V2, V3, V4 and V5 variants, led to an improvement in the nutritional quality of the forage compared to the mixture of pure grass stand, made in variant V1. Variants V3 and V5 stood out due to their superior nutritional profiles, having the highest protein content and the lowest fiber levels, indicating increased digestibility and forage value.

Variant V1, the mixture composed exclusively of grasses (*Lolium perenne*, *Dactylis glomerata* and *Festuca arundinacea*), showed the highest fiber and ADF (Acid Detergent Fiber) content, classifying it as the most fibrous and difficult-to-digest variant.

Variant V2 achieved the highest RFQ score (117.88), being the forage mixture with the best potential for rapid consumption and utilization by livestock.

The superior quality of V2 is attributed to the

inclusion of *Cicer arietinum*, which significantly bolstered the protein profile and digestibility. Furthermore, the diversification of the grassland with Lamiaceae species, such as *Ocimum basilicum* and *Dracocephalum moldavica*, alongside *Phacelia tanacetifolia* and *Nigella damascena*, contributed to a more balanced and functional forage.

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