COMPARATIVE STUDY ON DRY MATTER PRODUCTION AND NUTRITIONAL QUALITY OF SOME TYPES OF GRASSLAND FORAGE MIXTURES

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Abstract

The aim of this paper was to compare dry matter production and nutritional quality for different forage mixtures. The study was carried out in the experimental field of the Grassland Research and Development Institute Brașov, in 2022. Twelve grassland forage mixtures were analysed, with different proportions of perennial grass and legume species, recommended for use in three modes (grazing, mowing and mixed). Dry matter production was determined on three mowings, their sum being analyzed, and the quality of the forage obtained from each mixture was analysed as the average of the three mowings. Dry matter production ranged between 8.25 t/ha and 17.02 t/ha, and the ANOVA results showed significant differences (p<0.01) between the 12 forage mixtures. The highest CP content (18.28%) was recorded in the C1 mixture (ICDP – 60%G, 40%L) – use: mowing. Regarding CF, the highest contents (28.74%; 27.29%) were obtained in C6 (Versamax Hot&Dry – 80%G, 20%L) – use: mixed and C7 (Versamax Robust – 95%G, 5%L) – use: mixed. The highest DDM value (74.06%) was obtained in C12 (Zanandrea – 63%G, 37%L) – use: grazing, with a significant difference compared to the other mixtures. The similarity of the fodder mixtures was different on each mowing, both for dry matter production and for nutritional quality, this aspect being highlighted by Cluster analysis.

Keywords: *forage mixtures, dry matter, nutritional quality, Cluster analysis*

1. INTRODUCTION

Permanent meadows and pastures cover about 70% of the world's agricultural land and perform a key role in feeding ruminants and other herbivores that produce meat, milk, and dairy products (Niedbała et al., 2022; Kitczak et al., 2023)

Fodder crops play pivotal role in the agricultural economy of developing countries by providing cheapest source of feed for livestock. The evaluation of novel forages in new growing environments involves assessment of forage establishment, including morphological factors, dry matter yield and quality characterization (Chapman et al., 2008; Mohajer et al., 2013)

Multispecies mixture cultivation for forage production recently emerged as an attractive alternative to growing species in monocultures (Marquard et al., 2009; Finn et al., 2013) because plant species diversity has a positive interaction with biomass production (Favre et al., 2019; Tahir et al., 2022). Moreover, numerous studies have reported that increasing species diversity could improve nutrient use, stabilize biomass production and suppress weeds (Picasso et al, 2008; Sanderson et al, 2004). Species diversity offers complementarity, insurance and sampling effects which are key drivers for enhancing the biomass yield and stability of the mixtures (Yachi and Loreau, 1999; Tilman, 2002; Cong et al., 2018).

Grass-legume mixtures are preferred over pure-grass forage stands throughout the world because they often increase the total yields of herbage and protein and of fer balanced nutrition (Albayrak and Ekiz, 2005; ALBAYRAK et al., 2011). Mixtures offer several potential advantages over pure grasses or pure legumes. These advantages include the control of erosion, weed control and prolonged stand longevity (Casler, 1988).

Grass-legume mixtures tend to provide a superior nutrient balance and produce higher forage yields. However, grass-legume mixtures are more difficult to manage than monoculture pastures because of competition for light, water, and nutrients (Charles and Lehmann, 1989; Serin et al., 1998; Berdahl et al., 2001; Albayrak and Ekiz, 2005).

The most common legumes for forage production are alfalfa (Medicago sativa L.), white clover (Trifolium repens L.), and red clover (Trifolium pratense L.), while those of grasses are orchardgrass (Dactylis glomerata L.), tall fescue (Festuca arundinacea Schreb.), and perennial ryegrass (Lolium perenne L.). Alfalfa is a drought-tolerant and high-quality forage legume compatible with perennial grasses, enhances dietary protein supply for livestock and improves pasture soil health (Bhandari et al., 2020; Tahir et al., 2022)

Increased species diversity offers an insurance effect in which mixtures with more species guarantee that some species continue functioning when others fail (Yachi and Loreau, 1999; Tahir et al., 2022). However, many studies also reported that increasing the number of species in the mixture has no clear advantages in terms of biomass production or stability (Ashworth et al., 2018; Miyazawa et al., 2014).

Apart from yield benefits, forage quality has great importance for practical farming. The amount of biomass and energy contents increased with properly conducted agricultural managements and practices (Roman et al., 2021). The plant species diversity can also influence the quality of biomass yields. However, the results of studies concerning the effects of plant diversity on forage nutritional quality are ambiguous and the effects were often reported to be small (Deak et al., 2007; Khalsa et al., 2014; Ergon et al., 2017). Large effects of species diversity on nutritional quality were only reported when mixtures composed of one to four species provided that legumes are combined with grasses (Finn et al., 2013, Kirwan et al., 2007; Suter et al., 2015).

The aim of this paper was to compare dry matter production and nutritional quality for different forage mixtures.

2. MATERIALS AND METHODS

The study was carried out in 2022, in the experimental field of the Research-Development Institute for Grasslands Brașov, on a preluvosol soil type, characterized by an average humus content, slightly acidic pH and an average content of nitrogen, phosphorus and potassium (Table 1).

Specification	UN	Value
pH	ind	6,00
Humus	%	3,02
Sum of exchangeable bases, (SB)	me/100 g sol	28,90
Degree of saturation, (VAh)	%	84,30
Nitrogen index, (IN)	ind	2,54
Mobile Phosphorus, (P-ALc)	ppm	33,50
Mobile potassium, (K-AL)	ppm	126,00

Table 1. Agrochemical characteristics of the experimental field soil

The climate regime in the period October 2021 - September 2022 was characterized by higher average monthly temperatures compared to the multiannual average, but with quantitatively lower precipitation compared to the multiannual sum of precipitation. The average temperature recorded during the analysed period recorded a deviation of +1.3 °C compared to the multi-year average. The amount of precipitation recorded during the analysed period is 176.0 mm lower than the amount of multiannual precipitation.

The sum of the degrees of temperature recorded was 476° by the first mowing, 1216° by the second mowing, and 1024° by the third mowing. The average temperatures obtained varied from 10.3°C/day in the period up to the first mowing, up to 18.4°C/day and 18.6°C/day, in the period after the first mowing (Table 2).

Description	01/04 – 16/05	17/05 – 21/07	22/07 – 14/09
Sum of temperature	476 °C	1216 °C	1024 °C
Average temperature	10.3 °C	18.4 °C	18.6 °C

Table 2. Temperature evolution during the period of active vegetation

The amount of precipitation recorded until the first mowing was 108 mm, between the first and second cut was 121 mm and between the second and third cut was 145 mm. The average amount of precipitation varied from 1.8 mm/day between the first and second mowing, to 2.3 mm/day till the first mowing and 1.6 mm/day between the second and the third mowing (Table 3).

Description	01/04 – 16/05	17/05 – 21/07	22/07 – 14/09
Sum of rainfall	108 mm	121 mm	145 mm
Average rainfall	2.3 mm	1.8 mm	1.6 mm

Table 3. Evolution of precipitation during the active vegetation period

The data presented are part of the experience "Comparative study of some forage mixtures of meadows", carried out by I.C.D.P. Brașov, in which several grassland mixtures, each with a different species composition, were analysed. (Table 4) The experimental field contains 12 feed mixtures, each with 4 repetitions:

- **Recommended usage: mowing**
  - C1 - Variant 1 (ICDP – 60%G, 40%L)
  - C2 - Variant 2 (Cutmax Orig. – 85%G, 15%L)
  - C3 - Variant 3 (Cutmax Clover – 70%G, 30%L)
  - C4 - Variant 4 (Prima Sementi – 85%G, 15%L)
- **Recommended usage: mixed**
  - C5 - Variant 5 (ICDP – 60%G,40%L)
  - C6 - Variant 6 (Versamax Hot&Dry – 80%G, 20%L)
  - C7 - Variant 7 (Versamax Robust – 95%G, 5%L)
  - C8 - Variant 8 (Prima Sementi – 91%G, 9%L)
- **Recommended usage: pasture**
  - C9 - Variant 9 (ICDP – 70%G, 30%L) –
  - C10 - Variant 10 (Grazemax Hot&Dry – 90%G, 10%L)
  - C11 - Variant 11 (Prima Sementi – 87%G, 13%L)
  - C12 - Variant 12 (Zanandrea – 63%G, 37%L)

Fertilization of the experimental lot was carried out with chemical fertilizers $N_{150}P_{50}K_{50}$ active substance/ha. In 2022, three mowings were carried out.

In order to determine the qualitative parameters, a sample of green mass was collected from each experimental plot, 4 samples were collected from each experimental variant. The samples were dried and then ground with a Grindomix GM 200 mill and prepared for the analysis of chemical parameters.

The determination of the chemical parameters was carried out by the Near Infrared Spectroscopy (NIRS) method, the analysed parameters being: crude protein (CP), ash (ASH), crude fiber (CF), acid detergent fiber (ADF), lignin (ADL), neutral fiber detergent (NDF), digestibility of the dry matter (DDM) and the digestibility of the organic matter (DOM).

3. RESULTS AND DISCUSSION

Data on dry matter production for the first, second and third mowings of mixed fodder harvested in 2022 are presented in Table 4.

Mowings	**MIXTURES**												**Duncan Test**
	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	**C11**	**C12**	**(α=5%)**
First mowing	9.35 a	8.80 ab	8.47 abc	9.30 a	8.55 ab	5.87 efg	5.80 fg	7.62 cd	6.77 de	6.27 ef	8.22 bc	5.27 g	0.90 %
Second mowing	4.07 a	2.32 bcd	2.57 b	2.57 b	2.52 bc	1.87 de	1.72 e	2.07 cde	1.90 de	1.80 e	2.47 bc	1.67 e	0.45 %
Third mowing	3.60 a	1.67 d	2.47 bc	2.70 b	2.17 c	1.70 d	1.35 de	1.17 e	1.12 e	1.72 d	2.22 c	1.30 de	0.42 %
Sum	17.02 a	12.80 c	13.52 bc	14.57 b	13.2 c	9.45 ef	8.87 ef	10.88 d	9.80 de	9.80 de	12.93 c	8.25 f	1,24 %

Table 4. Dry matter production (t/ha) for different types of mixtures

The highest dry matter yields were obtained for the C1 mixture, for all three mowings, as well as for the sum of the mowings (17.02 t/ha), which showed significant differences compared to the other forage mixtures. The lowest dry matter yields, for the sum of the three mowings, were obtained for the C12 mixture (8.25 t/ha).

MIXTURES	**CP %**	**ASH %**	**CF %**	**ADF %**	**ADL %**	**NDF %**	**DDM %**	**DOM %**
C1	18.28a	10.31a	25.51cd	29.08cde	3.84abc	46.38c	68.79bcd	62.88e
C2	17.14bc	9.79abc	25.97bcd	29.55bcde	3.43bcde	49.44bc	69.16bc	63.68cde
C3	17.33b	10.44a	25.68cd	28.91de	3.58abcd	46.57c	69.03bc	63.95cde
C4	17.18bc	9.72abc	26.90bc	30.54abc	3.90ab	49.40bc	67.68cd	61.75e
C5	17.18bc	9.89ab	26.02bcd	28.87de	3.96a	46.23c	68.17bcd	62.60e
C6	16.04d	9.03c	28.74a	31.52a	3.17defg	53.68a	66.34d	62.73e
C7	16.37cd	9.29bc	27.29ab	30.79ab	2.93fg	54.59a	68.92bc	65.91bc
C8	16.89bcd	9.78abc	25.33d	28.75e	3.38cdef	48.08c	69.87bc	65.40bcd
C9	16.27cd	9.34bc	26.42bcd	30.37abcd	3.31def	52.53ab	68.12bcd	63.42de
C10	16.94bcd	9.73abc	25.98bcd	29.81bcde	2.98efg	51.59ab	70.52b	66.53b

C11	16.77bcd	9.82ab	25.87bcd	28.49ef	4.02a	47.03c	67.67cd	62.06e
C12	16.45bcd	9.48bc	23.30e	27.02f	2.74g	46.89c	74.06a	69.78a
Duncan Test (α = 5%)	0.94%	0.78%	1.50%	1.56%	0.47%	3.39%	2.48%	2.31%

Table 5. Nutritional quality for different types of feed mixtures

The average of the three mowings crude protein content (CP%) varied between 16,04% and 18,28%, placing the forage obtained in the quality classes from medium to excellent (Canbolat et al., 2006). According to the Duncan test, the fodder from C1 (ICDP – 60%G, 40%F) differed significantly from the other variants, with the highest protein content (18,28%). This was also due to the higher percentage of legumes in the composition of the mixture on this variant (40%). The mixtures recommended for use by mowing (variants 1-4) and variant 5, mixed use, stood out for their higher crude protein content (>17%) compared to the mixtures recommended for mixed use or grazing (Table 5).

The average mineral elements content (crude ash – ASH%) for the three mowing varied between 9,03% and 10,44%, values that fell in terms of the Duncan test in three homogeneity classes (a, b, c), the differences being significant for the 12 variants forage mixtures studied. The highest content of total mineral elements of the forage was obtained by the mixtures recommended to be used by mowing, C1 (10,31%) and C3 (10,44%) (Table 5).

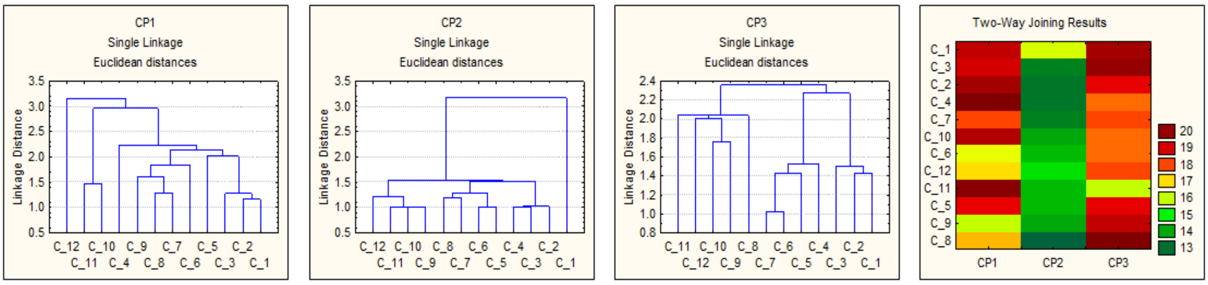
The average value of crude fiber content (CF%) varied between 23,30% and 28,74%. A crude fiber content between 20-30% characterizes a good quality forage, optimal for the ruminants forage ration (Nichita, 1984), thus the forage obtained from most variants is of good quality. From the perspective of the recommended use, the mixture recommended to be used by grazing, C12 obtained the lowest values of crude fiber content (23,30%), with a significant difference from the other variants.

Regarding the lignin content (ADL%) from forage mixtures, the values was between 2,74% and 4,02%. The lowest accumulation of lignin in the feed was recorded on C12 (2,74%), with positive influence because lignin is a major anti-nutritional component of grasses (Chaves et al., 2002a). Lignin limits cell wall (fiber) digestion by providing a physical barrier to microbial attack and the concentration of both fiber and lignin increases as plants mature (Van Soest 1978, Chaves et al. 2002b). Abundant data show negative correlations between the lignin concentration in plants and both dry matter (DM) and fiber digestibility (Jung et al. 1997).

The highest average values of the digestibility coefficients of the dry matter (DDM) were recorded in the forage mixtures were between 66,34% and 74,06%, C12 recorded the highest values (74,06%) with significant differences ($p \leq 0.05$), on the Duncan's test.

The digestibility coefficients of the organic matter (DOM) had the same evolution as the digestibility coefficients of the dry matter (DDM).

Using the technique of multivariate analysis by clustering, the relationship that existed between the 12 variants of fodder mixtures studied, from the point of view of crude protein content, for the three mowings, is presented in Fig. 1(a, b, c, d).



a) **b)** **c)** **d)**

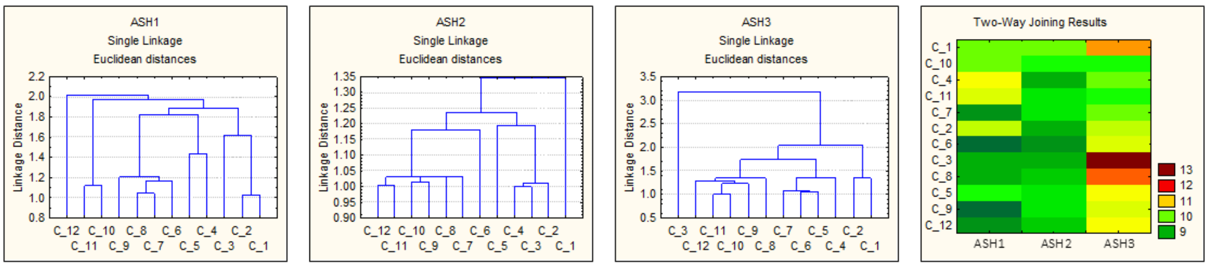
Fig. 1. Similarity analysis of feed mixtures for crude protein (CP) at:

a. First mowing, b. Second mowing, c. Third mowing, d. Synthesis

At the first mowing, the feed mixtures were grouped into three main subclusters. The first cluster consists of variants C10 and C11, mixtures recommended for grazing. The second cluster consists of variants C7 and C8, mixtures recommended for mixed. The third cluster consists of variants C1, C2 and V3, mixtures recommended for mowing.

At the second mowing, the feed mixtures were grouped into three main clusters, which grouped the 12 variants of feed mixtures according to the recommended use. Thus, the first cluster grouped the 4 mixtures recommended for grazing: C9, C10, C11 and C12. The second main cluster is represented by the feed mixtures recommended for mixed use: C5, C6, C7 and V8. The third cluster consists of the recommended forage mixtures for mowing: C2, C3 and C4, with the exception of the forage mixture C1, which was classified differently from the other 3 recommended for mowing mixtures. Variant C1, as can be seen from the two-way cluster analysis (Figure 1d) recorded the highest crude protein content (approximately 16%).

Cluster analysis, regarding the similarity of the 12 forage mixtures in terms of total ash content is found in Fig. 2 (a, b, c, d).



a) **b)** **c)** **d)**

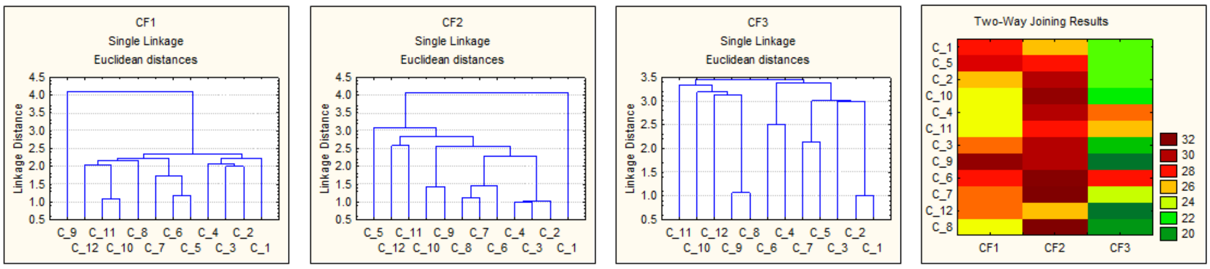
Fig. 2. Similarity analysis of feed mixtures for ash (ASH) at:

a. First mowing, b. Second mowing, c. Third mowing, d. Synthesis

At the first mowing, the feed mixtures were grouped into three subclusters, so the first subcluster was formed by C10 and C11, the second cluster by C7 and C8, and the third subcluster by C1 and C2.

At the second and third mowings, the feed mixtures were grouped differently following clustering, compared to the first harvest. At the third mowing, the feed mixture C3 was highlighted, which had a crude ash content of approximately 13% (Figure 2d).

In Figure 3 (a, b, c, d) the graphical representation of the cluster analysis, regarding the similarity of the 12 feed mixtures in terms of crude fiber content, at the three mowings can be found.



a) **b)** **c)** **d)**

Fig. 3. Similarity analysis of feed mixtures for crude fiber (CF) at:

a. First mowing, b. Second mowing, c. Third mowing, d. Synthesis

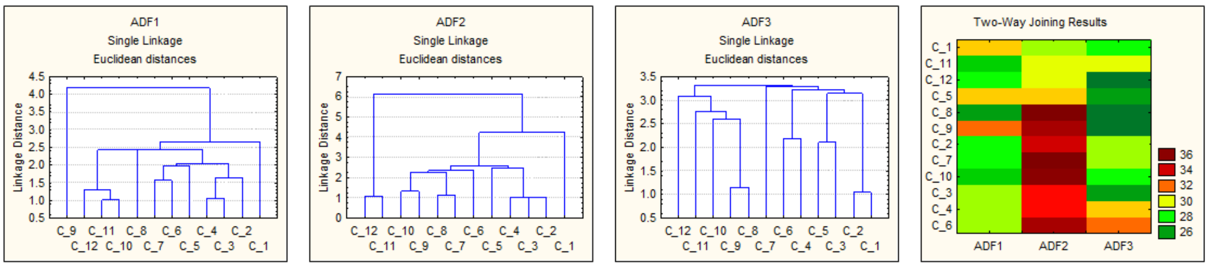
At the first mowing, the feed mixtures were grouped into two main clusters. The first cluster consisted of the feed mixtures recommended for mixed use (C5, C6, C7 and C8) and grazing (C10, C11 and C12). The exception to the characteristics of this cluster is feed mixture C9, which had the highest crude fiber content (approximately 30%) (Figure 3d).

The second cluster is composed of the feed mixtures recommended for mowing (C1, C2, C3 and C4) (Figure 3a), which had crude fiber contents ranging between 26-28% (Figure 3d).

At the second mowing, the feed mixtures analyzed were separated into four clusters. The first cluster and the second cluster include the mixtures recommended for grazing C12 and C11, and C10 and C9, respectively. The second cluster consists of three feed mixtures recommended for mixed use (C8, C7 and C6), and the third cluster consists of three feed mixtures recommended for mowing (C4, C3 and C2).

At the third mowing, as can be seen from Figure 3d, the crude fiber contents of the feed mixtures were lower, compared to the first two harvest harvests.

For the lignocellulose (ADF) content, in terms of the degree of similarity, which existed between the 12 feed mixtures studied, it is observed that in the case of the three mowings, approximately the same evolution was maintained as in the case of the crude fiber content of the feeds (Figure 4 a, b, c, d).

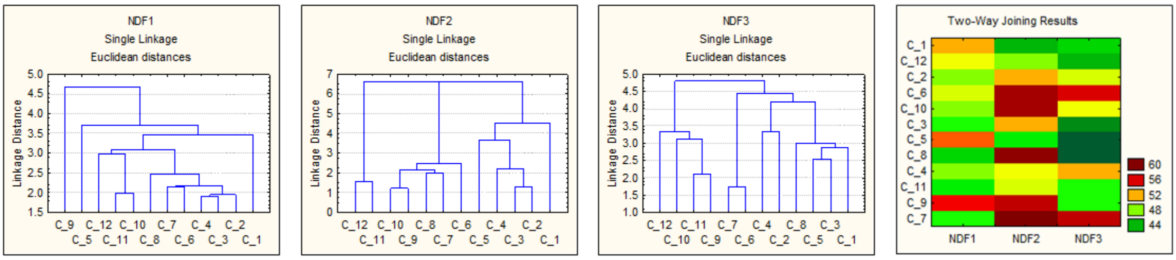


a) **b)** **c)** **d)**

Fig. 4. Similarity analysis of feed mixtures for ADF at:

a. First mowing, b. Second mowing, c. Third mowing, d. Synthesis

The cluster analysis, regarding the similarity of the 12 feed mixtures in terms of total NDF fiber content, of the three mowings are shown in Figure 5 a, b, c, d.



a) **b)** **c)** **d)**

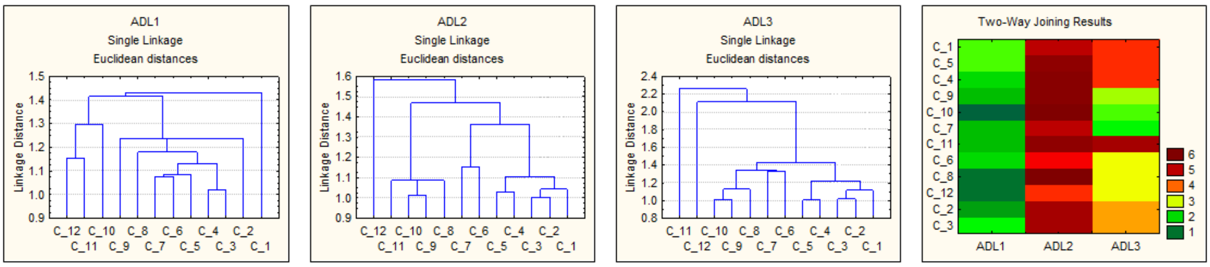
Fig. 5. Similarity analysis of feed mixtures for NDF at:

a. First mowing, b. Second mowing, c. Third mowing, d. Synthesis

Using cluster analysis, the relationship that existed between the 12 variants of fodder mixtures studied, in terms of lignin content, for the three mowings, which are presented in Figure 6 a, b, c.

The fodder mixtures were classified into two main clusters, at the first mowing, grouping three variants of mixtures recommended for grazing (C10, C11 and C12) and 8 variants of mixtures recommended for mixed use and mowing (C2, C3, C4, C5, C6, C7, C8 and C9).

From Figure 6d it is noted that the highest concentrations of lignin in fodder were recorded at the second mowing.

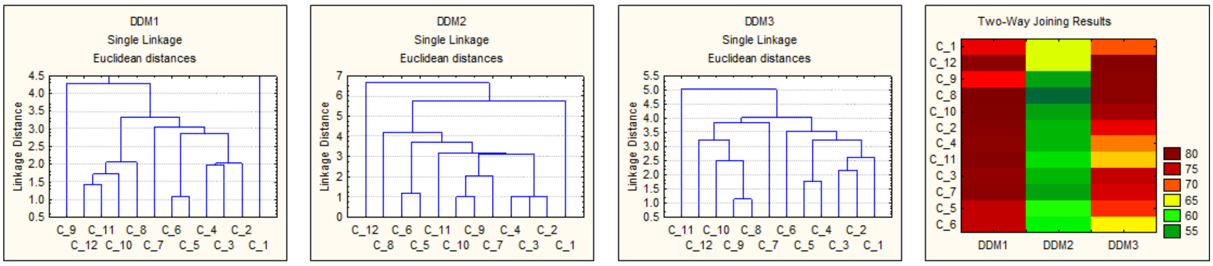


a) **b)** **c)** **d)**

Fig. 6. Similarity analysis of feed mixtures for ADL at:

a. First mowing, b. Second mowing, c. Third mowing, d. Synthesis

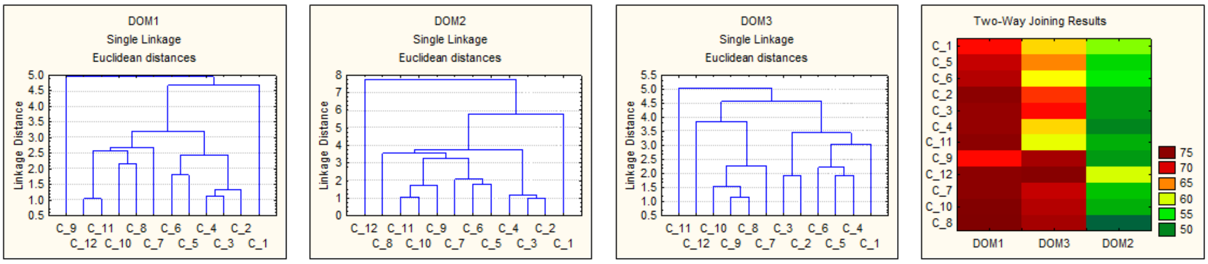
Regarding the digestibility of dry matter in the studied feed mixtures, from Figure 7d, it is noted that the lowest values were obtained in the second mowing, ranging between 55-65%, this being also due to a higher accumulation of lignin in the forages mowed in this harvest. The highest values were obtained in the first mowing.



a) b) c) d)

Fig. 7. Similarity analysis of feed mixtures for digestibility dry matter (DDM) at:
a. First mowing, b. Second mowing, c. Third mowing, d. Synthesis

In Figure 8 a, b, c, the graphical representation of the cluster analysis, regarding the similarity of the 12 feed mixtures in terms of the digestibility coefficients of organic matter, at the three harvesting times.



a) b) c) d)

Fig. 8. Similarity analysis of feed mixtures for digestibility organic matter (DOM) at:
a. First mowing, b. Second mowing, c. Third mowing, d. Synthesis

At the first mowing, the feed mixtures were grouped into two main clusters. The first cluster consists of variants C2, C3, C4, C5 and C6, mixtures recommended for mowing and mixed use. The second cluster consists of two mixtures recommended for mixed use C7 and C8 and three feed mixtures recommended for grazing use (C10, C11 and C12). The exception to the characteristics of these two clusters was the feed mixtures from ICDP C1 and C9, which recorded lower values (Figure 8d). At the second mowing, the grouping of feed mixtures in terms of digestibility coefficients was made into three clusters. Thus, the first cluster was composed of feed mixtures recommended for grazing: C9, C10 and C11. The second cluster is composed of feed mixtures recommended for mixed use C5, C6 and C7. The third cluster is composed of the recommended forage mixtures for mowing C2, C3 and C4.

At the third mowing, the forage mixtures were classified differently following clustering, compared to the first and second mowings.

4. CONCLUSIONS

The highest dry matter yields were obtained with the C1 forage mixture, both for all three mowings and for the sum of the mowings (17.02 t/ha), which registered significant differences compared to the other forage mixtures.

The average of the three mowings crude protein content (CP%) varied between 16.04% and 18.28%, placing the forage obtained in the quality classes from medium to excellent.

From the perspective of the recommended use, the mixture recommended to be used by grazing, variant C12 obtained the lowest values of crude fiber content (23.30%), with a significant difference from the other variants.

Regarding the degree of similarity, which existed between the 12 variants of mixtures studied, it was observed that from the point of view of the nutritional parameters analyzed (CP, CF, ASH, ADF, ADL, NDF, DDM and DOM) the feed mixtures were grouped according to the recommended method of use.

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