

Productivity and Vegetation Structure Evolution of Complex Mixtures of Perennial Forage Grasses and Leguminous Species, in the Conditions of the Center of Moldova, Romania

Margareta Naie¹, Oana Mîrzan^{1*}, Maria Diana Bărcan¹, Mihai Stavarache^{2*}, Simona Dumitriu², Elena Stavarache², Adriana Muscalu³, Diana Batîr Rusu⁴, Constantin Lungoci⁵

¹Agricultural Research - Development Station, Secuieni, Neamţ County, Romania

²Development Station - Meadows Development, Vaslui, Vaslui County, Romania

³National Institute of Research-Development for Machines and Installations Designed for Agriculture and Food Industry, Bucharest, District 1, Romania

⁴Plant Genetic Resources Bank "Mihai Cristea", Suceava, Suceava County, Romania

⁵Ion Ionescu la Brad - University of Life Sciences, Iaşi, Iaşi County, Romania

*Corresponding authors. E-mail: spanuoana@yahoo.com; stavarachem@scdpvs.ro

ABSTRACT

Research in our country on the number of species needed to set up temporary grasslands has highlighted the productive and structural superiority of complex mixtures of more than two species.

Although for the composition of mixtures is intended to use species and varieties as well as possible adapted to the environment, annual climatic variations sometimes influence the behaviour of the species and therefore productivity. The need to reduce doses of chemical fertilizers in the exploitation of temporary grasslands requires finding solutions to saturate the multiple ecological niches of temporary grassland ecosystems. These can be achieved by using a mix of a larger number of species adapted to the pedo-climatic conditions specific to the cultivation area, as well as by using an appropriate ratio between these species.

The purpose of the study was to follow the productivity and evolution of the vegetation structure in some crop mixtures, in different conditions of fertilization. The research was conducted under the conditions of the Center of Moldova from Romania at ARDS Secuieni - Neamţ, during the 2018-2021 period. The production of dry matter according to the type of mixture and the doses of fertilizers used the percentage of participation of perennial grasses and leguminous species in the vegetation structure, as well as its quality was analysed.

From the analysis of the obtained data, the highest feed production (24.82 Mg·ha⁻¹ DM) was obtained in the variant fertilized with N₈₀P₄₀ and sown with the mixture consisting of 70% grasses (*Festuca arundinacea* 30% + *Dactylis glomerata* 20% + *Festuca pratensis* 20%) and 30% leguminous (*Medicago sativa* 20% + *Trifolium pratense* 10%). At the first cut, in the mixture of 70% grasses (*Festuca arundinacea* 30% + *Dactylis glomerata* 20% + *Festuca pratensis* 20%) and 30% leguminous (*Medicago sativa* 20% + *Trifolium pratense* 10%), the participation percentage of grasses in the vegetation structure varied from 57% in the non-fertilized variant, to 61% in the variant fertilized with N₈₀₊₄₀P₄₀. The quality of the feed obtained was good for all variants studied.

Keywords: fertilization, vegetation structure, CP content, RFQ.

INTRODUCTION

Perennial grasses and leguminous species react differently to the application of chemical fertilizers. Thus, phosphate fertilizers stimulate leguminous species, and nitrogen fertilizers stimulate grasses species. Perennial leguminous species also procure the nitrogen needed to form their own proteins through symbiotic microorganisms and make some of it available to the grasses

with which they are mixed, leading to increased feed production and reducing the need for nitrogen-based chemical fertilizers. As a key element, nitrogen is a major factor for feed production and quality, but the time of harvest is also of particular importance in order to maintain a more balanced ratio between leaves and stems in plants (Schellberg et al., 1999; Le Bauer and Treseder, 2008; Karki et al., 2009; Zait et al. 2022). Fertilization with high doses of

nitrogen-based fertilizers, in addition to being expensive, can lead to the lush development of grasses species in mixtures and to almost pure grass crops, the leguminous species being gradually eliminated from the vegetation structure, especially for long-term used grasslands (Suding et al. 2005; Clark and Tilman, 2008; Bai et al., 2010; Vintu et al., 2010). In addition, many leguminous species have a well-developed root system and can make better use of soil water reserves, thus higher production can be obtained (Huyghe, 2003; Armstrong et al., 1999; Erice et al., 2010). Better genotypes of perennial grasses and leguminous species for feed used in mixtures play an important role in obtaining high-mineral quality production, with positive effects on animal husbandry and development (Herrmann et al., 2005; Schitea et al., 2007; Thumm, 2008; Nyfeler et al., 2009; Tomić et al., 2011; Dincă and Dunea, 2018; Petcu et al., 2019; Petcu et al., 2022).

After two years of use, in general, in the vegetation structure, changes in the grass-leguminous species ratio can be identified, compared to the sowing ratio. There is a decrease in the presence of plants from leguminous species, until their disappearance from the vegetation structure and an increase in the presence of plants from the various group (Barszczewski et al., 2007).

The proportion of participation in mixtures of perennial grasses and leguminous species depends on the use and duration of use of temporary grasslands and on the biological properties of the used species. For setting up temporary grasslands used as hayfields, high-waisted species with a close developmental rhythm are used, while medium-sized or low-sized species predominate for pastures, with different developmental rhythm, high speed growing and growing energy, resistance to soil compaction and high vivacity (Belesky et al., 2002; Sanderson et al., 2005). In the case of the mixtures it is necessary for it to form a well-concluded vegetation carpet, which will ensure in time a balanced development of the plants, for a long-lasting grassland, in the pedoclimatic conditions of the location (Butkutė and Daugėlienė, 2008).

The purpose of the study was to follow the productivity and evolution of the floristic in some complex mixtures, in different conditions of fertilization. The research was conducted within ARDS Secuieni - Neamț, Romania, in the 2018-2021 period, under the conditions of the Center of Moldova. The production of dry matter according to the type of mixture and the doses of fertilizers used, the percentage of participation of perennial grasses and leguminous species in the vegetation structure, as well as its quality was analyzed.

Five species of perennial grasses (*Dactylis glomerata* - Intensiv variety, *Bromus inermis* - Doina variety, *Lolium perenne* - Mara variety, *Festuca arundinacea* - Barelite variety, *Festuca pratensis* - Barvital variety) and four species of perennial leguminous (*Medicago sativa* - Mihaela variety, *Onobrychis viciifolia* - Splendid variety, *Lotus corniculatus* - Giada variety, *Trifolium pratense* - Rotrif variety) were used in mixtures to establish temporary grasslands.

MATERIAL AND METHODS

The stationary experience was located in the experimental field at the Secuieni-Neamț Agricultural Research - Development Station (ARDS Secuieni), located between the geographical coordinates of 46°51'15" northern latitude and 26°51'00" east longitude, in the S-E part of Neamț County. The soil is a typical faeosome kambic type, well supplied in phosphorus (77.6 ppm P_{AL}), calcium (13.6 meq·100⁻¹·g soil Ca), magnesium (1.8 meq/·100⁻¹·g soil Mg), medium supplied in nitrogen (16.2 ppm N-NO₃) and active humus (1.88%) and poorly supplied in potassium (124.6 ppm K₂O) with a slightly acidic pH (pH in H₂O - 5.98).

The climate of the experimentation area is of continental temperate type. During the analysed period (2017-2021 agricultural years), the climatic conditions were recorded by the unit's own weather station, located in the experimental field and recorded values with high variability. In terms of average air temperature, compared to the multiannual average, it was surplus. Thus, the average

monthly temperature was 10.3°C, compared to 8.9°C as the multiannual average, registering a deviation of 1.3°C. The average monthly temperatures, recorded by agricultural years, were: 10.2°C (in 2017-2018 agricultural year), 10.0°C (in 2018-2019 agricultural year), 11.2°C (in 2019-2020 agricultural year) and 9.9°C (in 2020-2021 agricultural year). Temperature deviations recorded per year were: 1.4°C (in 2017-2018 agricultural year), 1.1°C (in 2018-2019 agricultural year), 2.4°C (in 2019-2020 agricultural year) and 1.0°C (in 2020-2021 agricultural year).

In terms of precipitation, the multiannual average was 544.3 mm, and during the analysed period, by agricultural years were registered: in 2017-2018 agricultural year - 520.2 mm, in 2018/2019 agricultural year - 450.2 mm, in 2019/2020 agricultural year - 376.0 mm, in 2020/2021 agricultural year - 399.8 mm. The deviations recorded, compared to the multiannual average of precipitation, had values between -24.1 mm (in 2017/2018 agricultural year) and -168.3 mm (in 2019/2020 agricultural year), thus finding an increase in the deficit from one year to the next.

The results obtained were directly influenced by both the thermal regime and the rainfall regime throughout the research period.

At the Secuieni Agricultural Research - Development Station, Neamț County, in the spring of 2018, a bifactorial experience was established, placed according to the method of subdivided plots in 4 replications having the dimensions of a plot of 10 m², and by the lateral eliminations of 2 m² made according to the experimental technique, a harvest surface remained, of 8 m². The experience was type 4x5, and the use was for hay, with three production cuts each year of experimentation. The harvest of experience was done in the phenophase of spike emergence for grasses species and late bud for leguminous species. In the autumn, the last cut took place until the end of the first decade of October, to allow enough time for the plants to prepare for wintering. Factor A was fertilization and had four graduations:

a₁-N₀P₀ - control variant; a₂-N₄₀P₄₀; a₃-N₈₀P₄₀; a₄-N₈₀₊₄₀P₄₀, and factor B was the mixture of perennial grasses and leguminous species with five graduations: b₁ - 20% grasses + 80% leguminous (20% *Dactylis glomerata* L. + 80% *Medicago sativa* L.) - control variant; b₂ - 65% grasses + 35% leguminous (30% *Bromus inermis* Leyss + 35% *Dactylis glomerata* L. + 35% *Onobrychis viciifolia* Scop.); b₃ - 70% grasses + 30% leguminous (30% *Dactylis glomerata* L. + 40% *Lolium perenne* L. + 20% *Medicago sativa* L. + 10% *Lotus corniculatus* L.); b₄ - 70% grasses + 30% leguminous (30% *Festuca arundinacea* Schreb. + 20% *Dactylis glomerata* L. + 20% *Festuca pratensis* Huds. + 20% *Medicago sativa* L. + 10% *Trifolium pratense* L.); b₅ - 80% grasses + 20% leguminous (45% *Festuca pratensis* Huds. + 35% *Festuca arundinacea* Schreb. + 20% *Trifolium pratense* L.).

Fertilization with phosphate fertilizers was done in autumn, and nitrogen-based fertilizers were administered annually in early spring when starting in vegetation, except for the graduation N₈₀₊₄₀, whose nitrogen difference was administered after the first cut. The harvesting was done at a height of 4-5 cm from the soil surface, with the Bertolini mower.

The obtained productions were weighed on each variant, and samples were taken from the harvested biomass for the determination of the dry matter (DM) content and for qualitative analyses, respectively: crude protein content (CP), neutral detergent fibre content (NDF), acid detergent fibre content (ADF) and for the calculation of Relative Forage Quality (RFQ).

The determination of the floristic composition was made by collecting samples according to the gravimetric method from each plot, at each cut and the floristic evolution was followed by groups of species (grasses, leguminous and species from other botanical families).

RESULTS AND DISCUSSION

In the agricultural year 2017-2018, of the establishment of the experience, no production cuts were made, but the

experience was maintained by cleaning cuts. After the emergence of plants, due to the lack of precipitation immediately after sowing, the growth rate was slow.

In the three years of vegetation (2019, 2020 and 2021) the variants were harvested at the first cut on May 20. The second cut was made at a 43 days from cut I, and cut II at a 53 days from I.

Fertilization with mineral fertilizers positively influenced dry matter production, and the results obtained were different depending on the mixtures taken in the study as well as the perennial grasses and leguminous species in the composition of the mixtures.

Mixtures of perennial grasses and leguminous species had a good adaptation to the pedoclimatic conditions of ARDS Secuieni. Production obtained, on average for three years, in non-irrigated mode, were between 16.60 Mg·ha⁻¹ DM in the variant unfertilized and sown with the mixture *Dactylis glomerata* 30% + *Lolium perenne* 40% + *Medicago sativa* 20% + *Lotus corniculatus* 10% and 24.82 Mg·ha⁻¹ DM in the variant fertilized with N₈₀P₄₀ and sown with the mixture *Festuca arundinacea* 30% + *Dactylis glomerata* 20% + *Festuca pratensis* 20% + *Medicago sativa* 20% + *Trifolium pratense* 10%.

The results obtained at the first cut showed that the highest value of dry matter production, of 10.77 Mg·ha⁻¹ DM, was obtained in the variant *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35%, variant fertilized with N₈₀₊₄₀P₄₀. Compared to the control variant, it achieved very significant production increases. The lowest production, of 6.07 Mg·ha⁻¹ DM, was obtained in the unfertilized variant, sown with the mixture consisting of *Festuca pratensis* 45% + *Festuca arundinacea* 35% + *Trifolium pratense* 20%. Comparing the production obtained with that of the control variant (*Dactylis glomerata* 20% + *Medicago sativa* 80%), it was observed that this mixture showed a negative, very significant, production difference (Table 1).

At the second cut, the largest production obtained was by 11.20 Mg·ha⁻¹ DM in the variant fertilized with N₈₀₊₄₀P₄₀ and sown with the mixture *Festuca arundinacea* 30% +

Dactylis glomerata 20% + *Festuca pratensis* 20% + *Medicago sativa* 20% + *Trifolium pratense* 10%. In all other variants analysed, fertilized with doses of N₈₀P₄₀ and N₈₀₊₄₀P₄₀, it was observed that the differences recorded compared to the control variant were positive, very significant (Table 1).

At the third cut, productions decreased to half compared to the second cut, due to the precipitation deficit. The largest production, of 5.08 Mg·ha⁻¹ DM, was obtained in the variant sown with the mixture *Festuca arundinacea* 30% + *Dactylis glomerata* 20% + *Festuca pratensis* 20% + *Medicago sativa* 20% + *Trifolium pratense* 10%, fertilized with N₈₀₊₄₀P₄₀ and the lowest production, of 4.03 Mg·ha⁻¹ DM, was obtained in the variant sown with the mixture *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35% unfertilized (Table 1).

From the results obtained throughout the analysed period, it was found that it was noted with the largest production, of 24.82 Mg·ha⁻¹ DM, the variant sown with the mixture *Festuca arundinacea* 30% + *Dactylis glomerata* 20% + *Festuca pratensis* 20% + *Medicago sativa* 20% + *Trifolium pratense* 10%, fertilized with N₈₀P₄₀ (Table 1).

Compared to the control variant, at which a production of 17.63 Mg·ha⁻¹ DM was obtained, the differences of all variants studied and fertilized with mineral fertilizers were positive, very significant, except for the mixtures from the unfertilized variants sown with the mixture *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35% and *Dactylis glomerata* 30% + *Lolium perenne* 40% + *Medicago sativa* 20% + *Lotus corniculatus* 10% (Table 1).

Analysing the results obtained, it is highlighted that by increasing the nitrogen dose, the dry matter production of the feed also increases. The distribution of annual feed production by harvesting cuts is influenced by the cultivation mode, the harvesting regime and the grass and leguminous species in the mixtures. The average recorded data show an uneven distribution of production by harvest cuts, due to the deficit of precipitation, making the influence of this factor felt.

Table 1. The influence of the interaction between the mixture and fertilization on the average production of dry matter during the 2018-2021 agricultural period

Variant		Production average - 2018-2021 (Mg·ha ⁻¹ DM)			
		First cut	Second cut	Third cut	Total
a ₁ - N ₀ P ₀ (control)	b ₁ - <i>D.g.</i> 20%+ <i>M.s.</i> 80% (control)	6.77 ^C	6.71 ^C	4.15 ^C	17.63 ^C
	b ₂ - <i>B.i.</i> 30%+ <i>D.g.</i> 35%+ <i>O.v.</i> 35%	6.83	7.13 [*]	4.03	17.99
	b ₃ - <i>D.g.</i> 30%+ <i>L.p.</i> 40%+ <i>M.s.</i> 20%+ <i>L.c.</i> 10%	6.14 ^{ooo}	6.35 ^o	4.11	16.60 ^{oo}
	b ₄ - <i>F.a.</i> 30%+ <i>D.g.</i> 20%+ <i>F.p.</i> 20%+ <i>M.s.</i> 20%+ <i>T.p.</i> 10%	6.84	9.32 ^{***}	4.91 ^{***}	21.07 ^{***}
	b ₅ - <i>F.p.</i> 45%+ <i>F.a.</i> 35%+ <i>T.p.</i> 20%	6.07 ^{ooo}	8.35 ^{***}	4.40 [*]	18.82 ^{***}
a ₂ - N ₄₀ P ₄₀	b ₁ - <i>D.g.</i> 20%+ <i>M.s.</i> 80%	8.19 ^{***}	6.62	4.46 ^{**}	19.27 ^{***}
	b ₂ - <i>B.i.</i> 30%+ <i>D.g.</i> 35%+ <i>O.v.</i> 35%	9.21 ^{***}	8.51 ^{***}	4.27	21.99 ^{***}
	b ₃ - <i>D.g.</i> 30%+ <i>L.p.</i> 40%+ <i>M.s.</i> 20%+ <i>L.c.</i> 10%	7.92 ^{***}	6.68	4.12	18.72 ^{***}
	b ₄ - <i>F.a.</i> 30%+ <i>D.g.</i> 20%+ <i>F.p.</i> 20%+ <i>M.s.</i> 20%+ <i>T.p.</i> 10%	7.72 ^{***}	9.45 ^{***}	4.53 ^{***}	21.70 ^{***}
	b ₅ - <i>F.p.</i> 45%+ <i>F.a.</i> 35%+ <i>T.p.</i> 20%	7.29 ^{**}	9.48 ^{***}	4.57 ^{***}	21.34 ^{***}
a ₃ - N ₈₀ P ₄₀	b ₁ - <i>D.g.</i> 20%+ <i>M.s.</i> 80%	9.66 ^{***}	7.61 ^{***}	4.68 ^{***}	21.95 ^{***}
	b ₂ - <i>B.i.</i> 30%+ <i>D.g.</i> 35%+ <i>O.v.</i> 35%	10.73 ^{***}	9.53 ^{***}	4.88 ^{***}	25.14 ^{***}
	b ₃ - <i>D.g.</i> 30%+ <i>L.p.</i> 40%+ <i>M.s.</i> 20%+ <i>L.c.</i> 10%	8.93 ^{***}	7.38 ^{***}	4.76 ^{***}	21.07 ^{***}
	b ₄ - <i>F.a.</i> 30%+ <i>D.g.</i> 20%+ <i>F.p.</i> 20%+ <i>M.s.</i> 20%+ <i>T.p.</i> 10%	9.14 ^{***}	10.66 ^{***}	5.02 ^{***}	24.82 ^{***}
	b ₅ - <i>F.p.</i> 45%+ <i>F.a.</i> 35%+ <i>T.p.</i> 20%	8.80 ^{***}	10.49 ^{***}	4.66 ^{***}	23.95 ^{***}
a ₄ - N ₈₀₊₄₀ P ₄₀	b ₁ - <i>D.g.</i> 20%+ <i>M.s.</i> 80%	9.29 ^{***}	7.66 ^{***}	5.06 ^{***}	22.01 ^{***}
	b ₂ - <i>B.i.</i> 30%+ <i>D.g.</i> 35%+ <i>O.v.</i> 35%	10.77 ^{***}	8.95 ^{***}	4.50 ^{**}	24.22 ^{***}
	b ₃ - <i>D.g.</i> 30%+ <i>L.p.</i> 40%+ <i>M.s.</i> 20%+ <i>L.c.</i> 10%	8.72 ^{***}	7.66 ^{***}	4.67 ^{***}	21.05 ^{***}
	b ₄ - <i>F.a.</i> 30%+ <i>D.g.</i> 20%+ <i>F.p.</i> 20%+ <i>M.s.</i> 20%+ <i>T.p.</i> 10%	8.31 ^{***}	11.20 ^{***}	5.08 ^{***}	24.59 ^{***}
	b ₅ - <i>F.p.</i> 45%+ <i>F.a.</i> 35%+ <i>T.p.</i> 20%	7.66 ^{***}	10.73 ^{***}	4.15	22.54 ^{***}
LSD	0.5	0.36	0.32	0.21	0.61
	0.1	0.48	0.43	0.28	0.82
	0.01	0.63	0.57	0.36	1.08

When setting up temporary grasslands, the floristic composition used is maintained or evolved according to the stability of the climatic elements of the area. Thus, the number of species can be reduced and new species can appear from the soil seed reserve (weeds).

Analysing the data for each of the cuts, it was found that at the first cut, in the control variant, with mixture consisting of 20% grasses species (*Dactylis glomerata*) and 80% leguminous species (*Medicago sativa*), the percentage of participation of grasses species in the vegetation structure was between 42.8% in the unfertilized variant and 46.1% in the variant fertilized with N₄₀P₄₀. The percentage of participation in the vegetation structure of leguminous species was between 51.6% in the variant fertilized with N₄₀P₄₀ and 54.7% in the unfertilized variant (Figure 1).

At the mixture with 65% grasses species

(*Bromus inermis* 30% + *Dactylis glomerata* 35%) and 35% leguminous species (*Onobrychis viciifolia* 35%) the percentage of participation of grasses species in the vegetation structure was 66.4% in the unfertilized variant and 71.3% in the variants fertilized with N₄₀P₄₀ and N₈₀₊₄₀P₄₀. The percentage of participation in the vegetation structure of leguminous species was between 25.7% in the variant fertilized with N₈₀P₄₀ and 30.8% in the unfertilized variant. The percentage of participation of various species was between 1.2% and 3.0% (Figure 1).

At the mixture with 70% grasses species (*Dactylis glomerata* 30% + *Lolium perenne* 40%) and 30% leguminous species (*Medicago sativa* 20% + *Lotus corniculatus* 10%), the percentage of participation of grasses species in the vegetation structure was between 61.5% in the variant fertilized with N₄₀P₄₀ and 65.4% in the variant fertilized with N₈₀P₄₀. The percentage of

participation in the vegetation structure of leguminous species was between 32.1% in the variants fertilized with $N_{80}P_{40}$ and $N_{80+40}P_{40}$ and 37.0% in the unfertilized variant. The percentage of participation of various species was between 0 % and 4.2% (Figure 1).

At the mixture with 70% grasses species (*Festuca arundinacea* 30% + *Dactylis glomerata* 20% + *Festuca pratensis* 20%) and 30% leguminous species (*Medicago sativa* 20% + *Trifolium pratense* 10%) the

percentage of participation of grasses species in the vegetation structure was between 57.0% in the unfertilized variant and in the variant fertilized with $N_{80}P_{40}$ and 61.0% in the variant fertilized with $N_{80+40}P_{40}$. The percentage of participation in the vegetation structure of leguminous species was between 36.6% in the variant fertilized with $N_{80+40}P_{40}$ and 41.1% in the variant fertilized with $N_{80}P_{40}$. The percentage of participation of various species was between 1.9% and 6.1% (Figure 1).

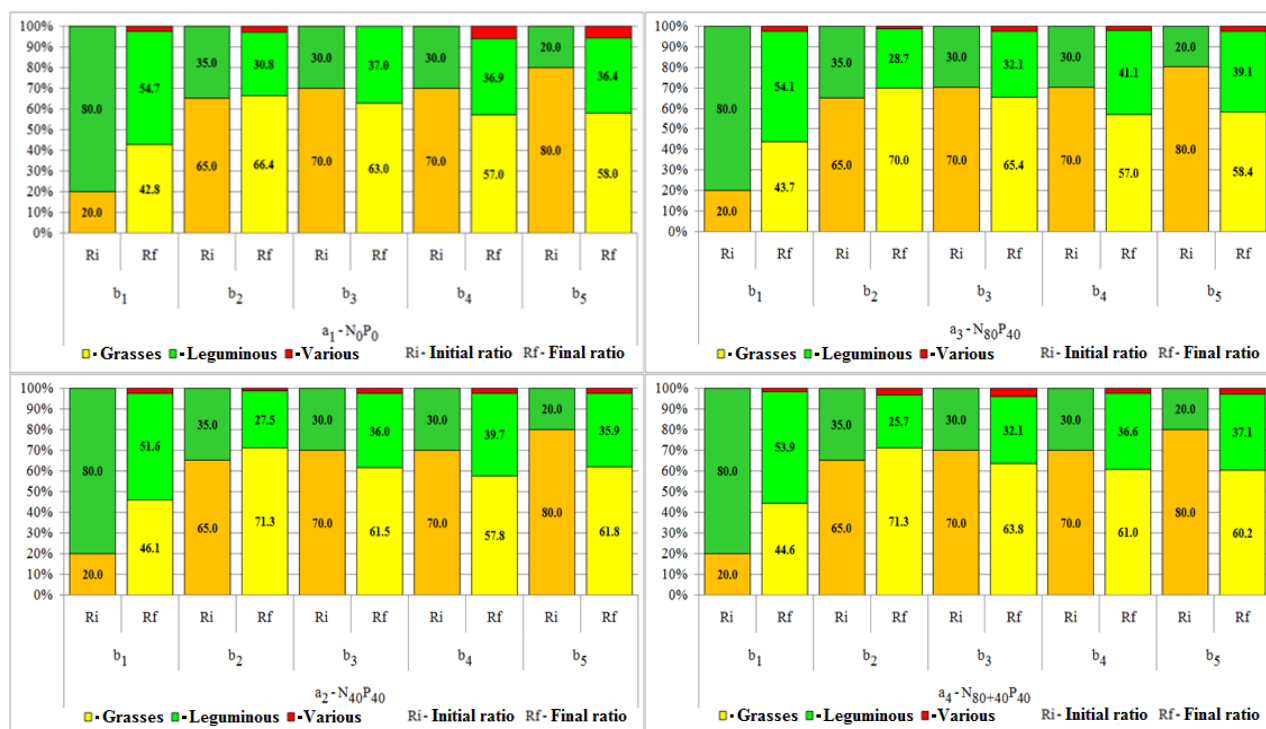


Figure 1. Influence of fertilization and mixture used on the vegetation structure, at the first cut, in the 2018-2021 agricultural period

At the mixture with 80% grasses species (*Festuca pratensis* 45% și *Festuca arundinacea* 35%) and 20% leguminous species (*Trifolium pratense* 20%) the percentage of participation of grasses species in the vegetation structure was between 58,0% (in the unfertilized variant) and 61.8% (in the variant fertilized with $N_{40}P_{40}$). The percentage of participation in the vegetation structure of leguminous species was between 35.9% (in the variant fertilized with $N_{40}P_{40}$) and 39.1% (in the variant fertilized with $N_{80}P_{40}$).The percentage of participation of various species was between 2.2% and 5.6% (Figure 1).

Analyzing the data obtained at the second cut, it was found that in the control mixture (*Dactylis glomerata* 20% + *Medicago sativa* 80%) the percentage of participation of grasses species in the vegetation structure was between 34.0% (in the variant fertilized with $N_{40}P_{40}$) and 41.4% (in the variant fertilized with $N_{80+40}P_{40}$). The percentage of participation in the vegetation structure of leguminous species was between 56.2% (in the variant fertilized with $N_{80+40}P_{40}$) and 59.1% in the variant fertilized with $N_{80}P_{40}$. The percentage of participation of various species was between 2.3% and 8.3% (Figure 2).

At the mixture with 65% grasses species

(*Bromus inermis* 30% + *Dactylis glomerata* 35%) and 35% leguminous species (*Onobrychis viciifolia* 35%) the percentage of participation of grasses species in the vegetation structure was between 39.8% in the variant fertilized with $N_{80+40}P_{40}$ and 44.0% in the unfertilized variant. The percentage of participation in the vegetation structure of leguminous species was between 53.1% in the variant fertilized with $N_{80+40}P_{40}$ and 57.5% in the variant fertilized with $N_{80+40}P_{40}$. The percentage of participation of various species was between 1.4% and 2.9% (Figure 2).

At the mixture with 70% grasses species (*Dactylis glomerata* 30% + *Lolium perenne* 40%) and 30% leguminous species (*Medicago sativa* 20% + *Lotus corniculatus* 10%), the percentage of participation of grasses species in the vegetation structure was between 44.3% in the variant fertilized with $N_{80}P_{40}$ and 46.6% in the variant fertilized with $N_{40}P_{40}$. The percentage of participation in the vegetation structure of leguminous species was between 49.2% in the variant fertilized with $N_{80+40}P_{40}$ and 54.0% in the variant fertilized with $N_{80}P_{40}$. The percentage of participation of various species was between 1.5% and 4.6% (Figure 2).

At the mixture with 70% grasses species (*Festuca arundinacea* 30% + *Dactylis glomerata* 20% + *Festuca pratensis* 20%) and 30% leguminous species (*Medicago sativa* 20% + *Trifolium pratense* 10%) the percentage of participation of grasses species in the vegetation structure was between 43.1% in the variant fertilized with $N_{80}P_{40}$ and 45.6% in the variant fertilized with $N_{80+40}P_{40}$. The percentage of participation in the vegetation structure of leguminous species was between 52.2% in the variant fertilized with $N_{80+40}P_{40}$ and 54.8% in the variant fertilized with $N_{80}P_{40}$. The percentage of participation of various species was between 2.1% and 2.9% (Figure 2).

At the mixture with 80% grasses species (*Festuca pratensis* 45% and *Festuca*

arundinacea 35%) and 20% leguminous species (*Trifolium pratense* 20%) the percentage of participation of grasses species in the vegetation structure was between 48.3% in the variant fertilized with $N_{80}P_{40}$ and 53.8% in the variant fertilized with $N_{80+40}P_{40}$. The percentage of participation in the vegetation structure of leguminous species was between 42.2% in the variant fertilized with $N_{80+40}P_{40}$ and 49.6% in the variant fertilized with $N_{80}P_{40}$. The percentage of participation of various species was between 2.0% and 4.0% (Figure 2).

Analyzing the data obtained at the third cut, it was found that in the control mixture (*Dactylis glomerata* 20% + *Medicago sativa* 80%) the percentage of participation of grasses species in the vegetation structure was between 39.2% in the variant fertilized with and 43.5% in the variant fertilized with $N_{80}P_{40}$. The percentage of participation in the vegetation structure of leguminous species was between 49.6% in the variant fertilized with $N_{80}P_{40}$ and 52.1% in the unfertilized variant and in the variant fertilized with $N_{40}P_{40}$. It was observed that the participation of grasses species in the vegetation structure increased, and that of leguminous species decreased. The percentage of participation of various species was between 6.9% and 9.1% (Figure 3).

At the mixture with 65% grasses species (*Bromus inermis* 30% + *Dactylis glomerata* 35%) and 35% leguminous species (*Onobrychis viciifolia* 35%) the percentage of participation of grasses species in the vegetation structure was between 50.0% in the unfertilized variant and 66.4% in the variant fertilized with $N_{40}P_{40}$. The percentage of participation in the vegetation structure of leguminous species was between 28.8% in the variant fertilized with $N_{40}P_{40}$ and 36.1% in the unfertilized variant. The percentage of participation of various species was between 4.8% and 13.9% (Figure 3).

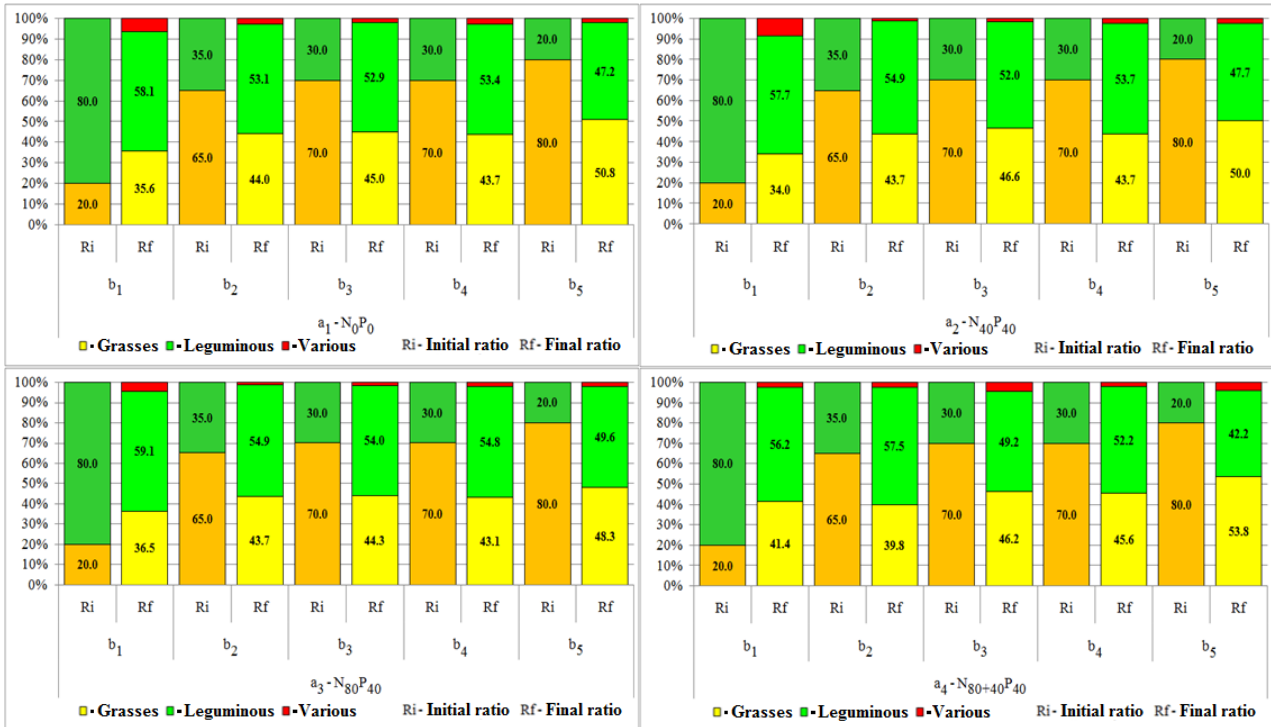


Figure 2. Influence of fertilization and mixture used on the vegetation structure, at the second cut, in the 2018-2021 agricultural period

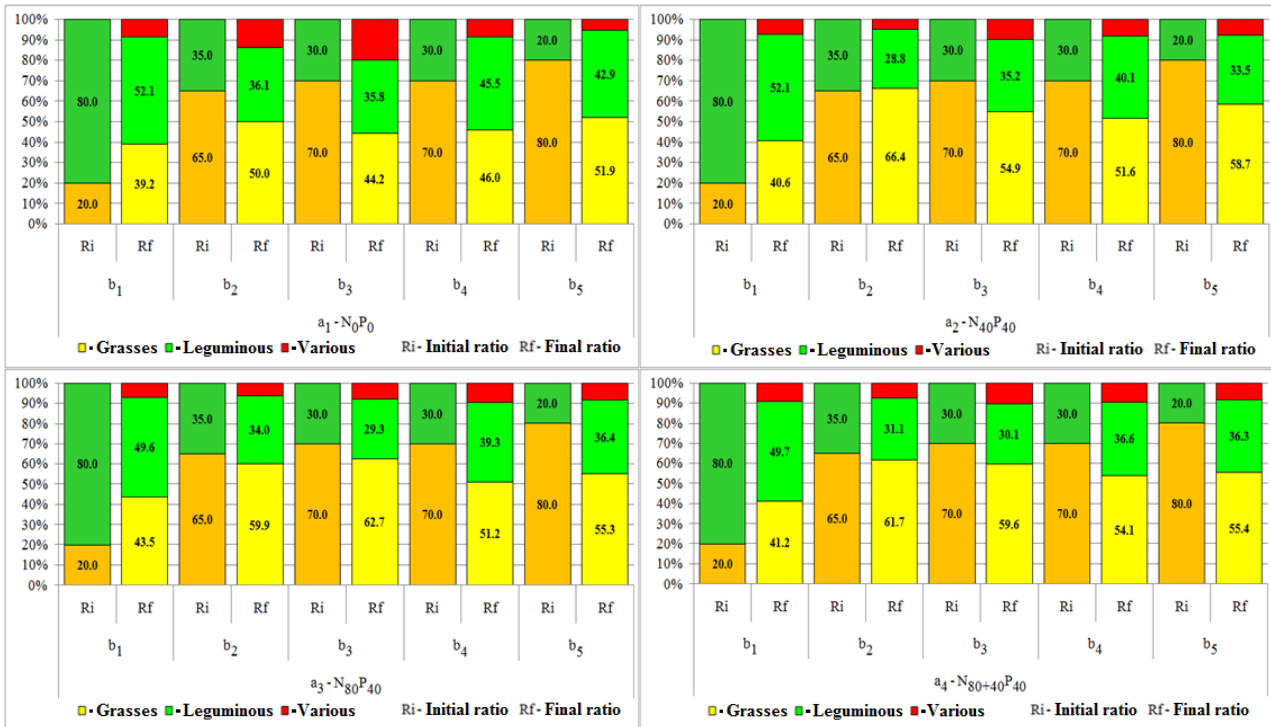


Figure 3. Influence of fertilization and mixture used on the vegetation structure, at the third cut, in the 2018-2021 agricultural period

At the mixture with 70% grasses species (*Dactylis glomerata* 30% + *Lolium perenne* 40%) and 30% leguminous species (*Medicago sativa* 20% + *Lotus corniculatus* 10%), the percentage of participation of

grasses species in the vegetation structure was between 44.2% in the unfertilized variant and 62.7% in the variant fertilized with $N_{80}P_{40}$. The percentage of participation in the vegetation structure of leguminous species

was between 29.3% in the variant fertilized with N₈₀P₄₀ and 35.8% in the unfertilized variant. The percentage of participation of various species was between 8,0% and 20.0% (Figure 3).

At the mixture with 70% grasses species (*Festuca arundinacea* 30% + *Dactylis glomerata* 20% + *Festuca pratensis* 20%) and 30% leguminous species (*Medicago sativa* 20% + *Trifolium pratense* 10%) the percentage of participation of grasses species in the vegetation structure was between 46.0% in the unfertilized variant and 54.1% in the variant fertilized with N₈₀₊₄₀P₄₀. The percentage of participation in the vegetation structure of leguminous species was between 36.6% in the variant fertilized with N₈₀₊₄₀P₄₀ and 45.5% in the unfertilized variant. The percentage of participation of various species was between 8.3% and 9.5% (Figure 3).

At the mixture with 80% grasses species (*Festuca pratensis* 45% + *Festuca arundinacea* 35%) and 20% leguminous species (*Trifolium pratense* 20%), the percentage of participation of grasses species in the vegetation structure was between 51,9% in the unfertilized variant and 58.7% in the variant fertilized with N₄₀P₄₀. The percentage of participation in the vegetation structure of leguminous species was between 33.5% in the variant fertilized with N₄₀P₄₀ and 42.9% in the unfertilized variant. The percentage of participation of various species was between 5.2% and 8.3% (Figure 3).

The quality of the feed obtained during the analyzed period was good in all the studied mixtures, being determined primarily by the doses of fertilizers administered, but also by the species and their proportion of participation in the sowing mixture.

Following the analysis of the influence of the interaction between fertilization and mixture used on the crude protein content of feed, it is observed that in conditions of non-fertilization, the control variant, respectively, the mixture *Dactylis glomerata* 20% + *Medicago sativa* 80% achieved the highest content, of 17.09 g·100 g⁻¹ DM, and the lowest, of 13.29 g·100 g⁻¹ DM was determined in the variant sown with the

mixture *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35% (Table 2).

From the results obtained, following the interaction between fertilization and mixture used, it is observed that from variants sown with mixtures *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35% and *Dactylis glomerata* 30% + *Lolium perenne* 40% + *Medicago sativa* 20% + *Lotus corniculatus* 10% a feed with similar crude protein values was obtained in all fertilized variants. All the differences obtained compared to the control variant were negative, very significant (Table 2).

Analyzing the interaction between fertilization and mixtures used, on the content of the feed in NDF, it was observed that the values obtained were between 42.42 g·100 g⁻¹ DM in the mixture *Dactylis glomerata* 30% + *Lolium perenne* 40% + *Medicago sativa* 20% + *Lotus corniculatus* 10%, unfertilized and 56.74 g·100 g⁻¹ DM in the mixture *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35%, fertilized with N₈₀₊₄₀P₄₀ (Table 2).

At the mixture *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35%, for all fertilization variants analyzed, the NDF highest content of feed was obtained, and all the differences obtained compared to the control variant were statistically assured, very significant.

Following the analysis of the influence of the interaction between fertilization and mixture used on the ADF content of feed, it is observed that from variant sown with mixture *Festuca pratensis* 45% + *Festuca arundinacea* 35% + *Trifolium pratense* 20%, under unfertilization conditions, the lowest ADF feed content was obtained, of 25.63 g·100 g⁻¹ DM, and the lowest, of 34.61 g·100 g⁻¹ DM was determined in the variant sown with the mixture *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35%, fertilized with N₈₀₊₄₀P₄₀ (Table 2).

In the case of variants fertilized with N₈₀P₄₀ and N₈₀₊₄₀P₄₀ the highest ADF

contents of the feed was obtained, of 34.35 g·100 g⁻¹ DM, respectively, 34.61 g·100 g⁻¹ DM, in variants sown with the mixture *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35%, and the differences obtained compared to the control variant (*Dactylis glomerata* 20% + *Medicago sativa* 80%) were positive, distinctly significant (Table 2).

From the results obtained it was found that the mixtures studied in unfertilization conditions registered the highest values of the relative quality of the feed (RFQ). Differences obtained between the control variant (*Dactylis glomerata* 20% + *Medicago sativa* 80%) and mixtures *Dactylis glomerata* 30% + *Lolium perenne* 40% + *Medicago sativa* 20% + *Lotus corniculatus* 10%, *Festuca arundinacea* 30% + *Dactylis*

glomerata 20% + *Festuca pratensis* 20% + *Medicago sativa* 20% + *Trifolium pratense* 10% and *Festuca pratensis* 45% + *Festuca arundinacea* 35% + *Trifolium pratense* 20%, were statistically assured, very significant. Thus the obtained feed falls into 0 quality class, respectively very good (according to Hancock, 2011) (Table 2).

In the case of *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35% mixture relative quality of the feed (RFQ) was 136 units, the difference from the control variant was negative, very significant, the obtained feed belonging to 1 quality class.

Analyzing the interaction between fertilization and mixtures used it is found that with increasing doses of mineral fertilizers, the relative quality of the feed decreases.

Table 2. The influence of the interaction between the mixture and fertilization on the forage quality, in the 2018-2021 agricultural period

Variant		Quality parameters			
		CP	NDF	ADF	RFQ
		g·100 g ⁻¹ DM			
a ₁ - N ₀ P ₀ (control)	b ₁ - <i>D.g.</i> 20%+ <i>M.s.</i> 80% (control)	17.09	46.08	30.85	144
	b ₂ - <i>B.i.</i> 30%+ <i>D.g.</i> 35%+ <i>O.v.</i> 35%	13.29 ⁰⁰⁰	49.29	30.33	136 ⁰⁰⁰
	b ₃ - <i>D.g.</i> 30%+ <i>L.p.</i> 40%+ <i>M.s.</i> 20%+ <i>L.c.</i> 10%	14.43 ⁰⁰⁰	42.42 ⁰⁰	27.12 ⁰⁰	166 ^{***}
	b ₄ - <i>F.a.</i> 30%+ <i>D.g.</i> 20%+ <i>F.p.</i> 20%+ <i>M.s.</i> 20%+ <i>T.p.</i> 10%	16.52	44.07	27.29 ⁰⁰	159 ^{***}
	b ₅ - <i>F.p.</i> 45%+ <i>F.a.</i> 35%+ <i>T.p.</i> 20%	16.65	43.22	25.63 ⁰⁰⁰	167 ^{***}
a ₂ - N ₄₀ P ₄₀	b ₁ - <i>D.g.</i> 20%+ <i>M.s.</i> 80%	16.17	48.37	32.14	135 ⁰⁰
	b ₂ - <i>B.i.</i> 30%+ <i>D.g.</i> 35%+ <i>O.v.</i> 35%	13.34 ⁰⁰⁰	53.21 ^{***}	32.57	122 ⁰⁰⁰
	b ₃ - <i>D.g.</i> 30%+ <i>L.p.</i> 40%+ <i>M.s.</i> 20%+ <i>L.c.</i> 10%	14.39 ⁰⁰⁰	45.34	28.80	152 [*]
	b ₄ - <i>F.a.</i> 30%+ <i>D.g.</i> 20%+ <i>F.p.</i> 20%+ <i>M.s.</i> 20%+ <i>T.p.</i> 10%	16.02	46.68	28.76	147
	b ₅ - <i>F.p.</i> 45%+ <i>F.a.</i> 35%+ <i>T.p.</i> 20%	15.97 ⁰⁰	46.18	27.00 ⁰⁰	153 ^{**}
a ₃ - N ₈₀ P ₄₀	b ₁ - <i>D.g.</i> 20%+ <i>M.s.</i> 80%	16.21	50.66 ^{**}	33.86 [*]	125 ⁰⁰⁰
	b ₂ - <i>B.i.</i> 30%+ <i>D.g.</i> 35%+ <i>O.v.</i> 35%	13.72 ⁰⁰⁰	56.33 ^{***}	34.35 ^{**}	111 ⁰⁰⁰
	b ₃ - <i>D.g.</i> 30%+ <i>L.p.</i> 40%+ <i>M.s.</i> 20%+ <i>L.c.</i> 10%	14.69 ⁰⁰⁰	48.08	30.17	140
	b ₄ - <i>F.a.</i> 30%+ <i>D.g.</i> 20%+ <i>F.p.</i> 20%+ <i>M.s.</i> 20%+ <i>T.p.</i> 10%	16.16	48.80	30.19	138 ^o
	b ₅ - <i>F.p.</i> 45%+ <i>F.a.</i> 35%+ <i>T.p.</i> 20%	16.11	47.65	28.07 ⁰⁰	146
a ₄ - N ₈₀₊₄₀ P ₄₀	b ₁ - <i>D.g.</i> 20%+ <i>M.s.</i> 80%	16.22	51.74	34.02 [*]	122 ⁰⁰⁰
	b ₂ - <i>B.i.</i> 30%+ <i>D.g.</i> 35%+ <i>O.v.</i> 35%	13.83 ⁰⁰⁰	56.74 ^{***}	34.61 ^{**}	110 ⁰⁰⁰
	b ₃ - <i>D.g.</i> 30%+ <i>L.p.</i> 40%+ <i>M.s.</i> 20%+ <i>L.c.</i> 10%	14.66 ⁰⁰⁰	48.01	30.07	140
	b ₄ - <i>F.a.</i> 30%+ <i>D.g.</i> 20%+ <i>F.p.</i> 20%+ <i>M.s.</i> 20%+ <i>T.p.</i> 10%	16.39	50.13 [*]	30.72	133 ⁰⁰⁰
	b ₅ - <i>F.p.</i> 45%+ <i>F.a.</i> 35%+ <i>T.p.</i> 20%	15.63 ⁰⁰	48.80	27.82 ⁰⁰	143
LSD	0.5	1.05	3.30	2.59	7
	0.1	1.40	4.42	3.47	9
	0.01	1.85	5.83	4.58	12

CONCLUSIONS

The results obtained showed that, under the conditions of the Center of Moldova in Romania, the feed productions obtained at

the studied crop mixtures were influenced by the species of mixture, the quantities of mineral fertilizers administered, as well as the climatic conditions of the agricultural year. Although the climatic conditions were

unfavourable, the feed production obtained was good for the unirrigated cultivation conditions, but in the years of experimentation only three cuts were performed.

Average production during the experimentation period (2018-2021), demonstrated that the best mixture was that constituted by 30% *Bromus inermis*, 35% *Dactylis glomerata* and 35% *Onobrychis viciifolia*, where the maximum level of production has been recorded in the variants fertilized with the doses of N₈₀P₄₀. Over these doses production began to decline mainly due to the inability of plants to use nutrients due to poor rainfall.

At the first cut, at all mixtures compared with the control variant, the grasses species dominated in the vegetation structure, at the second cut the ratio changed in favour of leguminous species to mixtures for three mixtures (b₂ - 30% *Bromus inermis* Leyss, 35% *Dactylis glomerata* L., 35% *Onobrychis viciifolia* Scop., b₃ - 30% *Dactylis glomerata* L., 40% *Lolium perenne* L., 20% *Medicago sativa* L., 10% *Lotus corniculatus* L. and b₄ - 30% *Festuca arundinacea* Schreb., 20% *Dactylis glomerata* L., 20% *Festuca pratensis* Huds., 20% *Medicago sativa* L. and 10% *Trifolium pratense* L.) and at the third cut grasses species dominated in the vegetation structure, in all mixtures.

REFERENCES

- Armstrong, R.D., Kuskopf, B.J., Millar, G., Whitbread, A.M., Standley, J., 1999. *Changes in soil chemical and physical properties following legumes and opportunity cropping on a cracking clay soil*. Anim. Prod. Sci., 39(4): 445-456.
- Bai, Y.F., Wu, J.G., Clark, C.M., Naeem, S., Pan, Q.M., Huang, J.H., Zhang, L.X., Han, X.G., 2010. *Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: evidence from inner Mongolia Grasslands*. Glob. Chang. Biol., 16(1): 358-372.
- Barszczewski, J., Wolicka, M., Burs, W., 2007. *Dynamic of changes in botanical composition of meadow sward in conditions of differentiated fertilisation and optimal moisture*. Grassland Science in Europe, 12: 114-117.
- Belesky, D.P., Fledhake, Ch.M., Boyer, D.G., 2002. *Herbage productivity and botanical composition of hill pasture as a function of clipping and site features*. Agronomy Journal, 94(3), U.S.A.
- Butkutė, R., and Daugėlienė, N., 2008. *Study on long-term meadow productivity and botanical composition in response to different liming and fertilization*. Biodiversity and Animal Feed 'Future Challenges for Grassland Production', 22, Sweden, ISBN 978-91-85911-47-9.
- Clark, C.M., and Tilman, D., 2008. *Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands*. Nature, 451: 712-715.
- Dincă, N., and Dunea, D., 2018. *On the assessment of light use efficiency in alfalfa (Medicago sativa L.) in the eco-climatic conditions of Târgoviște piedmont plain*. Rom. Agric. Res., 35: 59-69. <https://doi.org/10.59665/rar3509>
- Erice, G., Louahli, S., Irigoyen, J.J., Sanchez-Diaz, M., Avicé, J.-C., 2010. *Biomass partitioning, morphology and water status of four alfalfa genotypes submitted to progressive drought and subsequent recovery*. Journal of Plant Physiology, 167: 114-120.
- Hancock, D.W., 2011. *Using Relative Forage Quality to Categorize Hay*. The University of Georgia and Ft. Valley State University, CSSF048, available on-line: <http://www.caes.uga.edu/commodities/fieldcrops/forages/pubs/RFAQcategorization.pdf>.
- Herrmann, A., Kelm, M., Kornher, A., Taube, F., 2005. *Performance of grassland under different cutting regimes as affected by sward composition, nitrogen input, soil conditions and weather - a simulation study*. European Journal of Agronomy, 22: 141-158.
- Huyghe, C., 2003. *Les fourrages et la production de protéines*. In: Actes des Journées AFPP. Paris: Association Française pour la Production Fourragère: 3-16.
- Karki, U., Goodman, M.S., Sladden, S.E., 2009. *Nitrogen source influences on forage and soil in young southern-pine silvopasture*. Agric. Ecosyst. Environ., 131(1): 70-76.
- LeBauer, D.S., and Treseder, K.K., 2008. *Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed*. Ecology, 89(2): 371-379.
- Nyfelner, D., Huguenin-Elie, O., Suter, M., Frossard, E., Connolly, J., Lüscher, A., 2009. *Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding*. Journal of Applied Ecology, 46: 683-691.
- Petcu, E., Schitea, M., Drăgan, L., Băbeanu, N., 2019. *Physiological response of several alfalfa genotypes to drought stress*. Rom. Agric. Res., 36: 107-118. <https://doi.org/10.59665/rar3613>
- Petcu, V., Popa, M., Ciornei, L., Todirică, I.C., Popescu, G., Simion, P.S., Schitea, M., 2022. *Forage mixtures with Alfalfa cultivars, Perennial Grasses and Anethum Graveolens*. Lucrări Științifice, Seria Agronomie, Iași, 65(1): 96-99. <https://www.uaiasi.ro/revagrois/PDF/2022-1/paper/18.pdf>

- Sanderson, M.A., Soder, K.J., Muller, L.D., Klement, K.D., Skinner, R.H., Goslee, S.C., 2005. *Forage mixture productivity and botanical composition in pastures grazed by cattle*. *Agron. J.*, 97: 1465-1471.
- Schellberg, J., Mösel, B.M., Kühbauch, W., Rademacher, I.F., 1999. *Long-term effects of fertilizer on soil nutrient concentration, yield, forage quality and floristic composition of a hay meadow in the Eifel mountains, Germany*. *Grass Forage Sci.*, 54(3): 195-207.
- Schitea, M., Varga, P., Martura, T., Petcu, E., Dihoru, A., 2007. *New Romanian cultivars of alfalfa developed at NARDI Fundulea*. *Rom. Agric. Res.*, 24: 47-50.
- Suding, K.N., Collins, S.L., Gough, L., Clark, C.M., Cleland, E.E., Gross, K.L., Milchunas, D.G., Pennings, S., 2005. *Functional- and abundance-based mechanisms explain diversity loss due to N fertilization*. *PNAS*, 102(12): 4387-4392.
- Thumm, U., 2008. *Influence of site conditions on interspecific interactions and yield of grass-legume mixtures*. *Biodiversity and Animal Feed 'Future Challenges for Grassland Production'*, 22: 332-334.
- Tomić, Z., Bijelić, Z., Žujović, M., Simić, A., Kresović, M., Mandić, V., Marinkov, G., 2011. *Dry matter and protein yield of alfalfa, cocksfoot, meadow fescue, perennial ryegrass and their mixtures under the influence of various doses of nitrogen fertilizer*. *Biotechnology in Animal Husbandry*, 27(3): 1219-1226.
- Vîntu, V., Talpan, I., Ionel, A., Samuil, C., 2010. *Influence of mixture and fertilization on the behavior of some grasses and perennial legume species on temporary pastures in the Moldavian forest steppe*. *Romanian Journal of Grassland and Forage Crops*, 1: 81-91.
- Zaiț, T., Nazare, A.I., Samuil, C., Vîntu, V., 2022. *Species productivity research for Festuca arundinacea Schreb. and Trifolium pratense L. cultivated alone or in simple mixtures, in the first year of vegetation, under the conditions of the Moldavian forest-steppe*. *Romanian Journal of Grassland and Forage Crops*, 26: 9-14.