

CHEMICAL COMPOSITION, DIGESTIBILITY AND NUTRITIVE VALUE OF SOWN GRASSLANDS OF CEREALS AND LEGUME MEADOW GRASSES

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Abstract

The development of some species of perennial grasses forage crops in double mixtures with red clover was investigated in order to establish the quality and nutritive value of forage under mountain conditions. The dry matter of the mixture of *D. glomerata* L. with *Tr. pratense* L. was found to have the highest content of NDF, ADF and cellulose. The exceeded compared to the mean value of the traits was 6.0%, 8.0% and 12.5%, respectively. The mixture of *Ph. pratense* L. with *Tr. pratense* L. was shown to have the lowest content of fiber structural components (excluding ADL). The biomass had the highest in vitro dry matter digestibility. The values of the parameter were 2.3 to 7.0% higher than those of the other mixtures in the experiment. The plant mass of the two-component mixtures with *L. perenne* L. and *F. rubra* L. contained significantly higher amount of ADL in dry matter and had the highest values of GE.

Key words: perennial grass mixtures, detergent fibers, energy value.

INTRODUCTION

The sustainable agriculture systems in Bulgaria offer new ecological and environmentally friendly methods to improve the quality and feed value of the crop mass.

Perennial grass mixtures with hay or pasture direction are part of good agricultural practices in forage production with balanced composition and low cost (Churkova & Churkova, 2021; Lazarev & Tyazhkorob, 2022; Manevska-Tasevska et al., 2024). Mixed grasslands with high productivity and longevity require selection of species with ecological and combinative ability to maintain dynamic stability in the grassland (Elgersma & Sørensen, 2016). Association and competition (compatibility) between components are tailored to the biological and productive characteristics of species in specific agroecological conditions (Rusinovci et al., 2016; Bozhanska et al., 2024b). They also have significant effects in terms of optimal use of soil resources, water and solar energy (Duchini et al., 2018; Huss et al., 2022).

Mixed grasslands form a long-lasting vegetation mass, with conditions for weed reduction and an

increase in the relative proportion of useful grasses and legume forage crops affecting the evaluation, quality and nutritional value of forage (Naydenova & Vasileva, 2016).

In the mountainous regions of Bulgaria, forage grasses are most exposed to stressful abiotic effects with significant impact on their sustainability and productivity (Bozhanski et al., 2023). Some of the most suitable species for the establishment of temporary grasslands (two-, three- or four-component mixtures) evaluated on the traits of productivity, longevity and quality are the species: *Trifolium pratense*, *Trifolium repens*, *Lotus corniculatus*, *Festuca pratensis*, *Festuca rubra*, *Dactylis glomerata* and *Phleum pratense* (Naydenova & Mitev, 2015; Churkova, 2019; Katova, 2023). The main structurally determining component in the composition of grass associations is perennial meadow grasses, which maintain a high relative share in the total forage mass, and legume species positively affect the stability and yield of both fresh and dry matter (Mitev & Naydenova, 2015; Naydenova et al., 2015). Ecologically, members of the *Fabaceae* family have the ability to supply nitrogen to grasses through symbiotic nitrogen fixation,

respectively increasing crude protein content and improving dry matter digestibility (Pirhofer-Walz et al., 2012; McElroy et al., 2017). The maturity stage is an important factor that influences the nutritional value of the forage plant, as the content of cell walls (including the values of lignified vascular and sclerenchymal tissue in the plant) affects the feed digestibility ratio (Valente et al., 2016). The concentration of those substances is closely related morphological structure of a plant and to habitat conditions (Sosnowski et al., 2017).

In this sense, in the conditions of the Central Balkan Mountain (Bulgaria), the mixed grassland of *Lotus corniculatus* with *Festuca rubra* is distinguished by its biologically determined resistance and suitability to variations in environmental factors (Mitev et al., 2010). The crude protein, crude fiber and nitrogen-free extractable substances in the dry matter of plant mass were 147.87 g kg⁻¹, 372.20 g kg⁻¹ and 306.30 g kg⁻¹, respectively (Churkova & Churkova, 2020). The mixture is highly productive and practically validated for the Central Balkan Mountain region (Bozhanska, 2017b; Churkova, 2019). The two-component mixture of *Trifolium repens* with *Poa pratensis*, grown under mountain conditions, has optimal conversion, low fiber content and high digestibility of dry biomass. The values of neutral-detergent fibers, acid-detergent fibers, cellulose and *in vitro* dry matter digestibility of the grasses were 356.5 g kg⁻¹ (NDF), 226.4 g kg⁻¹ (ADF) and 192.4 g kg⁻¹ (cellulose) and 712.6 g kg⁻¹ (IVDMD), respectively (Bozhanska, 2017a). In the experimental area, good compatibility was also found between the components in mixed grasslands of *Lolium perenne* with *Trifolium repens*, *Lotus corniculatus* L. and *Trifolium pratense* L. The biomass formed was of high nutritional value, and dry matter digestibility ranged from 622.18 g kg⁻¹ to 642.54 g kg⁻¹ (Petkova et al., 2022).

Cell wall components are a factor in forage quality and a major source of energy for animals (Katova & Naydenova, 2017a, 2017b; Ivanova et al., 2019).

The aim of the present study was to determine the fibers cell wall components, *in vitro* dry matter digestibility and energy nutritional value of forage biomass obtained from two-component mixtures of *Trifolium pratense* L.

with *Festuca rubra* L., *Lolium perenne* L., *Dactylis glomerata* L. and *Phleum pratense* L. grown under mountain conditions.

MATERIALS AND METHODS

The experiment was conducted in the period 2020-2023, in the experimental field of the Research Institute of Mountain Stockbreeding and Agriculture of Troyan (Bulgaria).

The objective of the study refers to four perennial species of grass forage (*Festuca rubra* L., *Lolium perenne* L., *Dactylis glomerata* L., *Phleum pratense* L.) grown in mixtures with red clover (*Trifolium pratense* L.) in a ratio of 50%:50%, under nonirrigated conditions.

The experimental variants included:

1. *Festuca rubra* L. + *Trifolium pratense* L. (FR + TrP);
2. *Lolium perenne* L. + *Trifolium pratense* L. (LP + TrP);
3. *Phleum pratense* L. + *Trifolium pratense* L. (PP + TrP);
4. *Dactylis glomerata* L. + *Trifolium pratense* L. (DG + TrP).

Sowing was done manually, scattered. The sowing rates of the studied forage species were calculated based on 100% seed germination. Immediately after sowing, the sown areas were rolled for better contact of the seeds with the soil and to ensure simultaneous germination of the plants. The plot size was 5 m², laid out in 4 replications.

The grasslands were mowed at the beginning of the tasseling/ear formation period for grasses and the bud-formation period/blossoming period for legumes. The weed control during the vegetation was mechanical, intending to not allow additional chemical intervention on the plants.

Indicators of study

The fibrous structural elements in the plant cell are analyzed in laboratory: Neutral Detergent Fibers (NDF, g kg⁻¹ DM), Acid detergent fiber (ADF, g kg⁻¹ DM) and Acid detergent lignin (ADL, g kg⁻¹ DM) by the Van Soest & Robertson (1979) detergent assay and *in vitro* dry matter digestibility (IVDMD) according to a two-way pepsin-cellulase method of Aufrere (1982). The polyosides are empirically calculated: Hemicellulose (g kg⁻¹ DM) = NDF -

ADF and Cellulose (g kg⁻¹ DM) = ADF - ADL. The lignification degree is expressed as the percentage of ADL and NDF.

The nutritional value of the feed has been estimated by the Bulgarian system as Feed Unit for Milk (FUM, in kg DM) and Feed units for growth (FUG, in kg DM) and calculated on the basis of equations according to the experimental values of Crude protein (CP), Crude fiber (CF), Crude fat (Cft) and nitrogen-free extract substances (NFE), recalculated by Todorov (2010) digestibility ratios: Gross energy (GE, MJ/kg DM) = 0.0242*CP + 0.0366*Cft + 0.0209*CF + 0.017*NFE - 0.0007*Zx and Exchangeable energy (EE, MJ/kg DM) = 0.0152*DP (Digestible protein) + 0.0342*Dft (Digestible fat) + 0.0128*DF (Digestible fibers)

+ 0.0159*DNFE (Digestible Nitrogen-free extractable substances) - 0.0007*Zx.

Soil and climatic characteristics in the experimental area

Temperature, humidity and the amount of macronutrients in the soil indirectly influence forage quality parameters through the amount of nutritions reaching the plant roots (Mariotte et al., 2020).

The soils in the area of experience are one of the poor in the country, making them unfavorable for agricultural crops because of their acidic (pH_{H2O}: 5.2-5.3; pH_{KCL}: 4.3-4.4) reaction and insufficient water-air regime (Table 1). Penkov (1988) found them suitable for growing forage grasses because they provide high productivity.

Table 1. Agrochemical soil analysis (0-40 cm)

Soil characteristics	pH		Σ N - NH ₄ +NO ₃	P ₂ O ₅	K ₂ O	Humus
Soil layer	H ₂ O	KCL	mg/kg ⁻¹	mg/100		%
0-20	5.3	4.4	20.2	2.4	9.9	1.44
20-40	5.2	4.3	8.6	1.2	5.9	0.96

The soils in the experimental area are light gray, pseudopodzolic. The content of the main nutrients in the soil layer was: from 0-20 cm - total N - 20.2 mg/1000 g, P₂O₅ - 2.4 mg/100 g, K₂O - 9.9 mg/100 g, humus - 1.44% and from 20-40 cm - total N - 8.6 mg/1000 g, P₂O₅ - 1.2 mg/100 g, K₂O - 5.9 mg/100 g, humus - 0.96% (Bozhanska, 2017a).

Considering the soil characteristics in the experimental area, we applied in autumn in the year of sowing: 15 kg P₂O₅/da (triple superphosphate) and in spring - 12 kg N/da (NH₄NO₃).

Temperature and precipitation are among the most important factors affecting forage yield and quality because they directly influence plant growth, community composition, nutrient concentration in plant tissues, and forage digestibility (Marshall et al., 2005).

In the years of the experiment, temperature values during the vegetation period (March-October) ranged from 14.6°C (2021) to 16.3°C (2023) for a 30-year average of 14.8°C. Rainfall totals (March-October) in the year of establishing the grasslands were normal for the area (550.2 mm versus 625.6 mm) and spring moisture created optimum conditions for the formation of the first undergrowth. In the second, third, and fourth trial years, growing

season precipitation totals were lower by 102 mm (2022) to 245.7 mm (2023) compared to the 30-year period (625.6 mm). Drought occurred in all seasons of the years of the experiment, which affected physiological processes during different phenophases and stages of individual development of the studied forage crops.

Experimental data were averaged and statistically processed using a variance analysis ANOVA, LSD at P = 0.05.

RESULTS AND DISCUSSIONS

The content of fiber structural components of cell walls is variable and increases with maturity stage in plants (Jung & Deetz, 1993).

In the first experimental year, *Phleum pratense* L. with *Trifolium pratense* L. had the lowest content of neutral-detergent fibers (415.0 g kg⁻¹), acid-detergent fibers (216.4 g kg⁻¹), acid-detergent lignin (28.6 g kg⁻¹) and cellulose (187.8 g kg⁻¹) (Table 2).

This mixture was characterized by the highest relative proportion of legume to wheat (legumes:grasses - 30.3%:13.6%) compared to the other grasslands studied (Bozhanska et al., 2024a) and the highest dry matter digestibility (713.2 g kg⁻¹).

In contrast, the biomass of the two-component mixture *Dactylis glomerata* L. + *Trifolium pratense* L. recorded the highest concentration of ADF (309.9 g kg⁻¹), ADL (37.9 g kg⁻¹) and cellulose (272.0 g kg⁻¹), which correlated positively with the low *in vitro* dry matter digestibility (627.6 g kg⁻¹).

The composition and characteristics of polysaccharides vary greatly among forages and plant cell walls (Li, 2021).

Neutral-detergent fibers is a laboratory indicator to predict the free uptake of feed by animals and the composition of acid-detergent fiber influences dry matter digestibility.

Table 2. Main structural fiber components of cell walls and *in vitro* dry matter digestibility (g kg⁻¹ DM) of mixed perennial grasslands with *Trifolium pratense* L., by year and average over the period

Variants	NDF	ADF	ADL	Hemicell	Cellul	IVDMD of Aufreere
2020						
FR + TrP	448.5	287.8	36.7	160.7	251.1	653.7
LP + TrP	452.4	236.8	32.1	215.6	204.7	698.4
PP + TrP	415.0	216.4	28.6	198.6	187.8	713.2
DG + TrP	433.4	309.9	37.9	123.5	272.0	627.6
2021						
FR + TrP	462.1	296.6	49.7	165.5	246.9	653.4
LP + TrP	472.0	258.3	43.7	213.7	214.6	677.5
PP + TrP	378.1	245.0	32.9	133.1	212.2	685.1
DG + TrP	440.9	296.5	26.6	144.4	269.9	654.2
2022						
FR + TrP	468.2	301.6	50.1	166.6	251.5	643.2
LP + TrP	465.6	271.4	44.8	194.2	226.6	661.4
PP + TrP	398.2	261.1	41.9	137.2	219.1	677.1
DG + TrP	451.6	297.5	31.0	154.1	266.5	651.3
2023						
FR + TrP	471.9	290.2	58.7	181.7	231.5	613.3
LP + TrP	468.4	262.9	49.0	205.5	213.9	633.5
PP + TrP	401.5	271.8	40.2	129.7	231.6	658.9
DG + TrP	586.2	280.2	35.2	306.0	245.0	621.1
2020-2023						
FR + TrP	462.7	294.1	48.8	168.6	245.3	640.9
LP + TrP	464.6	257.4	42.4	207.3	215.0	667.7
PP + TrP	398.2	248.6	35.9	149.6	212.7	683.6
DG + TrP	478.0	296.0	32.7	182.0	263.4	638.6
Mean	450.9	274.0	39.9	176.9	234.1	657.7
LSD_{0.05}	57.6	24.5	8.9	75.4	21.5	21.6
Significance of the differences in the values of the indicator over the years	P < 0.05	P < 0.01	P = 0.01	P > 0.05	P < 0.001	P < 0.01
Significance of differences in indicator values between variants	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P < 0.01

The results of the analysis showed that in the second and third growing seasons, the mixture of *Phleum pratense* L. with *Trifolium pratense* L. had the lowest contents of NDF (378.1 and 398.2 g kg⁻¹, respectively), ADF (245.0 and 261.1 g kg⁻¹, respectively), hemicellulose (133.1 and 137.2 g kg⁻¹, respectively) and cellulose (212.2 and 219.1 g kg⁻¹, respectively).

The mixtures of *Trifolium pratense* L. with *Lolium perenne* L. (472.0 g kg⁻¹) and *Festuca rubra* L. (468.2 g kg⁻¹) had the highest concentration of total fiber components (NDF). The excess in the values of the indicator is 2.1-19.9% (for 2021) and 0.6-15.0% (for 2022). The low values of ligno-cellulose complexes have a significant effect on the digestibility of

biomass of the grassland. In the years of the experiment, the mixture *Phleum pratense* L. + *Trifolium pratense* L. had the highest *in vitro* dry matter digestibility. On average, the values of the parameter (683.6 g kg^{-1}) were higher by 2.3 to 7.0% compared to the other grasses in the experiment. According to Junga et al. (2012) high digestibility of plant forage had a positive effect on ruminant productivity. A 1% improvement in trait values can increase animal growth by 10% (Naydenova, 2009). Data from the analysis showed a significant difference ($P < 0.01$) in digestibility of two-component mixtures both by year and by variant. Statistical proved the difference in the values of the indicator between the mixtures of *Phleum pratense* L. and *Lolium perenne* L. with those of *Dactylis glomerata* L. and *Festuca rubra* L.

It has been found that as plants mature, the concentration of lignin fraction increases and is associated with decreased cell wall digestibility (Jung & Deetz, 1993). The low levels of the indicator are associated with a biological feature of *Dactylis glomerata* L. and when evaluating the quality of forage with legumes, its values range from 2.98% to 4.00% (Naydenova et al., 2015).

In the years of the experiment, *Dactylis glomerata* L. was the dominant species in the mixed grasslands with *Trifolium pratense* L. (occupying up to 77.7% in the grassland) (Bozhanska et al., 2024), which mainly influenced the percentage of structural components in the dry matter. The acid-detergent lignin content in the biomass of the two-component mixture (*Dactylis glomerata* L. + *Trifolium pratense* L.) ranged from 26.6 g kg^{-1} to 37.9 g kg^{-1} , with an average of 32.7 g kg^{-1} . The values of the indicator compared with the other variants studied were lower in the individual vegetations (the exception being the first vegetation) by 23.7 to 86.8% (for 2021), 35.2 to 61.6% (for 2022) and 14.2 to 66.8% (for 2023), respectively. For the experimental period, the mixed grassland also had the highest cellulose content (a structural polyside that performs a supporting function in plant cells and is partially digestible by ruminants). The obtained results indicate a decrease in the values of the indicator from the first to the fourth growing season, when the age and the proportion of grasses in the grassland increase

(Bozhanska et al., 2024a). The species *Dactylis glomerata* L. was also dominant in the undergrowth of mixed grasslands with *Lotus corniculatus* L., *Onobrychis adans* and *Trifolium repens* L. (Naydenova et al., 2015).

In the later stages of forage crop vegetation, a slow increase in acid-detergent fibers and hemicellulose content occurs, followed by a decrease in the amount of minerals and the relative proportion of digestible cell contents (Carrier et al., 2011). In this regard, according to the analysis of the experimental data, in the second, third and fourth experimental years, *Festuca rubra* L. grasses with *Trifolium pratense* L. showed maximum values concerning the parameters acid-detergent fibers and acid-detergent lignin. The high content of the components negatively affected the digestibility of the grassland. With the increase in plant age, the forage mass in the variant had the lowest *in vitro* dry matter digestibility, namely 653.4 g kg^{-1} (2021), 643.2 g kg^{-1} (2022) and 613.3 g kg^{-1} (2023). The percentage difference in the values of the indicator for the three years, between the studied two-component mixtures ranged from 1.1 to 7.4%.

Forage quality, expressed by the fiber characteristics of the plant cell and digestibility, has a significant influence on animal productivities.

Averaged over the period, the mixture *Dactylis glomerata* L. + *Trifolium pratense* L. had the highest content of structural fiber components, namely: neutral-detergent fibers (478.0 g kg^{-1}), acid-detergent fibers (296.0 g kg^{-1}) and cellulose (263.4 g kg^{-1}). The excess over the mean value of the traits studied was 6.0%, 8.0% and 12.5%, respectively. In contrast, the biomass in the *Phleum pratense* L. + *Trifolium pratense* L. mixture was shown to have lower levels of NDF, ADF and cellulose compared to the mean maximum values of the indicators.

A significant difference in the concentration of acid-detergent lignin was found between the two-component mixtures of *Dactylis glomerata* L. + *Trifolium pratense* L. (32.7 g kg^{-1}) and *Phleum pratense* L. + *Trifolium pratense* L. (35.9 g kg^{-1}) with the mixtures of *Festuca rubra* L. + *Trifolium pratense* L. (48.8 g kg^{-1}) and *Lolium perenne* L. + *Trifolium pratense* L. (42.4 g kg^{-1}). Studies also showed a proven difference in the amount of cellulose between the

grasslands of *Dactylis glomerata* L. + *Trifolium pratense* L. (263.4 g kg⁻¹) and *Festuca rubra* L. + *Trifolium pratense* L. (640.9 g kg⁻¹) with those of *Lolium perenne* L. + *Trifolium pratense* L. (215.0 g kg⁻¹) and *Phleum pratense* L. + *Trifolium pratense* L. (212.7 g kg⁻¹). There was no statistically significant difference (at P < 0.05) between the treatments with respect to hemicellulose content. The mean values ranged from 149.6 g kg⁻¹ (*Phleum pratense* L. + *Trifolium pratense* L.) to 207.3 g kg⁻¹ (*Lolium perenne* L. + *Trifolium pratense* L.).

Over the four-year period of the experiment (considering the sources of variation - years and variants), the environmental conditions had a significant effect on the amount of the major fiber structural components of the cell walls

(NDF – P < 0.05, ADF – P < 0.01, ADL – P = 0.01 and cellulose – P < 0.001) in the dry matter of the two-component mixtures studied.

The biological process of lignification is estimated to be a major factor limiting the nutritional value of forage and inhibiting its digestibility (Akin & Chesson, 1989; Casler & Jung 2006). In the years of the experiment, the coefficients by degree of lignification in mixed grasslands ranged from 8.2% to 12.4% (2020), from 7.1% to 10.5% (2021), from 6.9% to 10.5% (2022) and from 6.0% to 8.7% (2023), respectively (Figure 1). As the age of the grasslands increased, a decrease in the indicator values was observed only in the two-component mixtures of *Lolium perenne* L. (by 2.7-4.8%) and *Dactylis glomerata* L. (by 4.0-6.4%).

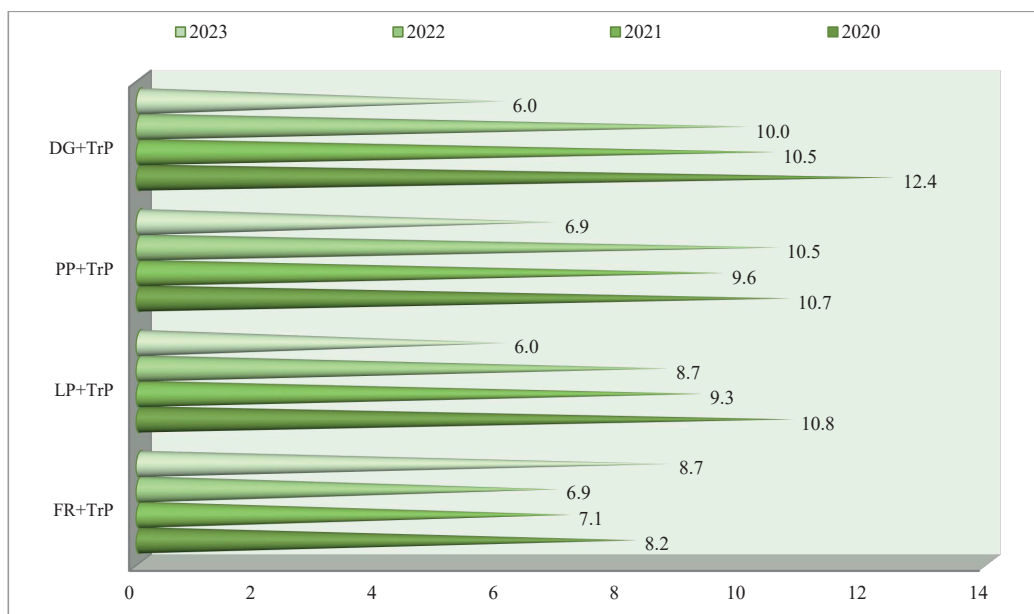


Figure 1. Lignification degree (coefficient) of perennial meadow grasses in mixed grasslands

Efficient utilization and evaluation of mixed grasslands are closely related to the analysis of cell wall components and energy nutritional value of the forage. The degradation of the fiber fraction in the dry matter is a major energy source for ruminants, and an accurate assessment of the calorific value is largely based on the balance between qualitative and

quantitative parameters in the forage of the variants studied.

Average over the period, the higher energy biomass was characterized by mixed grasslands of *Festuca rubra* L. with *Trifolium pratense* L. (19.04 MJ/kg DM) and of *Lolium perenne* L. with *Trifolium pratense* L. (19.10 MJ/kg DM) (Table 3).

Table 3. Potential energy nutrient value of mixed perennial grasslands with *Trifolium pratense* L., by years and average over the period

Variants	GE	EE	FUM	FUG
2020				
FR + TrP	19.02	8.14	0.75	0.67
LP + TrP	19.06	8.28	0.76	0.69
PP + TrP	18.63	8.52	0.79	0.73
DG + TrP	18.92	8.30	0.76	0.70
2021				
FR + TrP	19.08	7.68	0.70	0.62
LP + TrP	19.54	7.88	0.71	0.63
PP + TrP	19.27	7.93	0.72	0.64
DG + TrP	19.48	7.84	0.71	0.63
2022				
FR + TrP	19.31	7.65	0.69	0.61
LP + TrP	19.05	8.07	0.74	0.66
PP + TrP	18.90	8.44	0.78	0.71
DG + TrP	18.86	7.71	0.70	0.62
2023				
FR + TrP	18.74	7.59	0.69	0.61
LP + TrP	18.76	7.77	0.71	0.63
PP + TrP	18.57	7.47	0.68	0.60
DG + TrP	18.25	7.40	0.67	0.60
2020-2023				
FR + TrP	19.04	7.77	0.71	0.63
LP + TrP	19.10	8.00	0.73	0.65
PP + TrP	18.84	8.09	0.74	0.67
DG + TrP	18.88	7.81	0.71	0.64
Mean	18.97	7.92	0.72	0.65
SD	0.12	0.15	0.02	0.02

Legend: GE (MJ/kg DM), EE (MJ/kg DM), FUM (in kg DM), FUG (in kg DM).

The amount of gross energy in the two-component mixtures exceeds the mean by 0.4 and 0.7%, respectively. In the variants of *Trifolium pratense* L. with *Phleum pratense* L. and *Dactylis glomerata* L., the values of the parameter were 18.84 MJ/kg DM and 18.88 MJ/kg DM, respectively.

The plant mass of the mixtures *Phleum pratense* L. + *Trifolium pratense* L. (8.09 MJ/kg DM) and *Lolium perenne* L. + *Trifolium pratense* L. (8.00 MJ/kg DM) was distinguished by higher values in terms of the amount of energy involved in the metabolic processes of the animals.

The exceeded over the mean value of the trait was 1.0-2.1%. The net energy nutrient content of the forage mass, expressed in terms of growth and milk feed units in both treatments, showed a slight excess in the FUM values (by 1.4-2.8%) compared to the mean (0.72 in kg DM).

In the grasslands of *Trifolium pratense* L. with *Festuca rubra* L. (7.77 MJ/kg DM) and *Dactylis glomerata* L. (7.81 MJ/kg DM), the metabolizable energy content had lower values compared to the mean. This trend was also

maintained in terms of the number of straw units (FUM and FUG) in dry matter. The empirically calculated values concerning the number of feed units for growth exceed the average value of the indicator by 3.1% only in the mixture of *Trifolium pratense* L. with *Phleum pratense* L. (0.67 in kg DM).

According to the analysis of the data in the study, there was no significant difference in the values of the indicators determining the energy-nutrient value of the sown perennial cereal grasses with *Trifolium pratense* L. (by year and average over the period). A similar trend was observed in the study of two-component mixtures of *Lotus corniculatus* L. with *Dactylis glomerata* L., *Festuca rubra* L. and *Phleum pratense* L. (Churkova & Churkova, 2020). Perennial grasses and legume grown in mixed grasslands are characterised by a specific morphological and physiological structure (Schaub et al., 2020). Their efficient utilisation and nutritional value are closely related to the analysis of cells walls components.

In the studied mixed grasslands, a correlation was found between the amount of energy regulating metabolic processes in the animal

organism and the number of feed units for growth with the digestibility of the biomass formed (Figure 2).

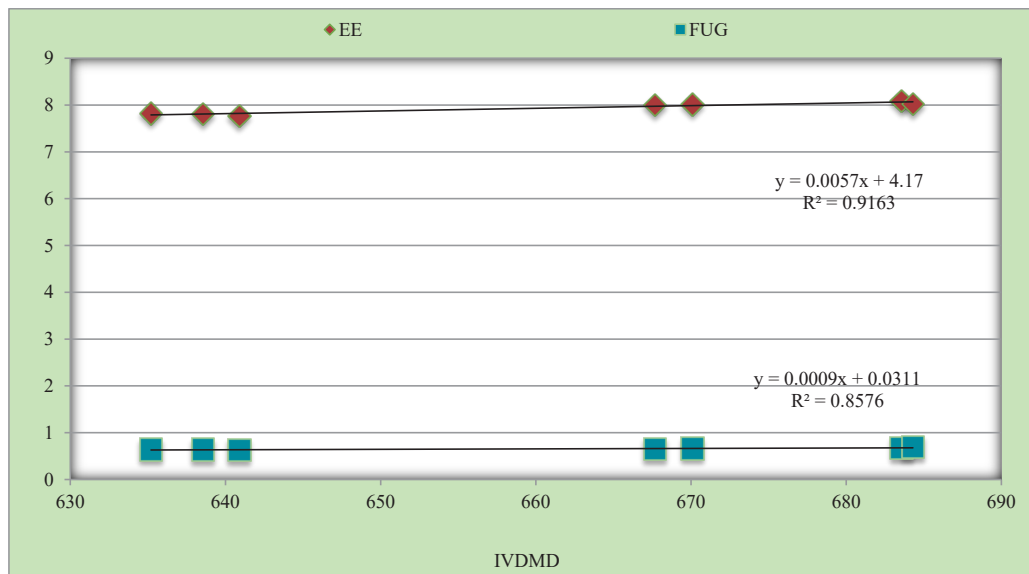


Figure 2. Regression relationship between *in vitro* dry matter digestibility (g kg⁻¹) with the amount of metabolizable energy (MJ/kg DM) and the number of feed units for growth (in kg DM) in mixed stands of perennial species with *Trifolium pratense* L.

The theoretical regression lines and the equations of regression dependence between the indicators EE и FUG с IVDMD are: $y = 0.0057x + 4.17$ (at determination coefficient $R^2 = 0.9163$) and $y = 0.0009x + 0.0311$ (at determination coefficient $R^2 = 0.8576$) with a level of significance $P < 0.05$.

The established regression models reveal a good opportunity for approximate determination of the energy value of forage in mixed grasslands through the digestibility of dry matter.

CONCLUSIONS

The mixture of *Dactylis glomerata* L. with *Trifolium pratense* L. had the highest content of neutral-detergent fibers (478.0 g kg⁻¹), acid-detergent fibers (296.0 g kg⁻¹) and cellulose (263.4 g kg⁻¹). The exceeded compared to the mean value of the traits was 6.0%, 8.0% and 12.5%, respectively. On average over the study period, the two-component mixture had the lowest *in vitro* dry matter digestibility (638.6 g kg⁻¹), and the lowest concentration of acid-detergent lignin (32.7 g kg⁻¹).

In contrast, mixed grasslands of *Phleum pratense* L. + *Trifolium pratense* L. were shown to have a lower detergent fibers content. For the study period, the variant biomass had the highest *in vitro* dry matter digestibility (683.6 g kg⁻¹), and the excess in the values of the parameter ranged from 2.3 to 6.6%. The two-component mixture also had the highest exchangeable and net (FUM and FUG) energy content.

The mixtures of *Trifolium pratense* L. with *Festuca rubra* L. and *Lolium perenne* L. were distinguished by a significantly higher content of acid-detergent lignin in the dry matter and by the highest gross energy values.

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