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CRUDE PROTEIN YIELD AND ENERGY NUTRITIONAL VALUE OF FODDER OF PERENNIAL GRASS MIXTURES

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Abstract

The aim of the study was to determine the amount of crude protein yield and the yield of feed units in the fodder biomass of six variants of two-component legume-grass mixtures (Lotus corniculatus - Festuca rubra; Trifolium repens - Lolium perenne; Trifolium repens - Poa pratensis; Trifolium pretense - Phleum pretense; Medicago sativa - Dactylis glomerata; Trifolium pretense - Festuca pratensis) that would provide high-quality fodder in the conditions of the Central Balkan Mountain. The highest crude protein yield is for the mixtures Tr. pratense L. + F. pratensis L. and Tr. repens L. + P. pratensis L. The legume crop predominated in the regrowths of both variants during the entire experimental period from 7.4% to 48.4%, respectively, and the ratio of legume:grass in the mixed grass stands was 47.7:40.3% (Tr. pratense L. - F. pratensis L.) and 62.3:13.9% (Tr. repens L.- P. pratensis L.). A proven difference in crude protein yield was found between Tr. repens L. - P. pratensis L. and M. sativa L. - D. glomerata L. mixtures. The highest yield of feed units for milk and growth was found in the forage mixture of Tr. pratense L. with Ph. pratense L.

Key words*: crude protein; feed unit for milk; feed unit for growth; grass-legume mixtures.*

INTRODUCTION

The plant development and the relationships between species in fodder crops within mixed crops are a significant factor in the composition and quality of the grass mass, and the agro-climatic conditions in the growing region affect their productive capabilities (Das et al., 2016; Gasiev et al., 2019). In this sense, the species selection, the morphological and physiological features, and the biochemical processes related to them are essential for the nutritional value of the obtained fodder (Pavlov, 1996).

Cultivation of perennial legume and grass crops with high adaptive and productive potential leads to a permanent increase (by 1.5-2.0 times more) in the quality and quantity of obtained fodder (Kurgak, 2010).

On the other hand, multicomponent mixed grass stands use soil resources more efficiently (Lipińska et al., 2018; Butenko et al., 2019; Churkova and Churkova, 2020) through the improved vertical arrangement of the root mass in the soil horizons, which allows a more complete assimilation of nutrients and formation of longer lasting and highly productive grass stands (Vasileva and Vasilev, 2012; Luscher et al., 2014; Kusvuran et al., 2014).

Perennial grass-legume and grass associations are a source of environmentally friendly fodder mass to meet animal food requirements (Chand et al., 2022). The composition of the grass stands, the digestibility of the crude protein, the fat, the amount of the fiber fraction and the nitrogen-free extractives are decisive for the energy nutritional value of the dry fodder mass. The inclusion of legume components in mixed grass stands is an effective method to increase the values of crude protein in dry matter (Ding et al., 2014; Cordeau et al., 2017; Churkova and Churkova, 2021; Naydenova et al., 2022) which are involved in the equations for predicting the energy nutritional value of fodder. The precise establishment of energy nutrition, which is the main criterion for modern evaluation of the quality of fodder determined by the fodder units for milk and growth (Todorov, 2010) is essential in the evaluation of fodder.

The aim of the study was to determine the amount of crude protein yield and the yield of feed units for milk and growth in the fodder biomass of six variants of two-component

grass-legume mixtures that would provide high-quality fodder in the conditions of the Central Balkan Mountain (Bulgaria).

MATERIALS AND METHODS

The study was conducted at the Research Institute of Mountain Stockbreeding and Agriculture, Troyan (Bulgaria), in the period 2014-2016, on light gray, pseudopodzolic soils, with pH_{KCL} = 4.3.

In the first experimental year (2014), the average precipitation amounts for the period of March-October were significantly higher (1046 mm) than normal for the region (664 mm), and in the third year (2016) by 10.4% lower. The spring regrowths of the first and third experimental years were well supplied with a sufficient moisture amount. In the second experimental year (2015), the growth of grass and legume crops took place under significantly drier conditions and at a higher average monthly air temperature for the period of March-October (15.9°C) compared to the values of the indicator for the first and third experimental year (14.8°C).

Experimental variants were:

1. *Lotus corniculatus* L. (cv. Leo) - *Festuca rubra* L. (cv. Ryder);
2. *Trifolium repens* L. (cv. Huia) - *Lolium perenne* L. (cv. Belida);
3. *Trifolium repens* L. (cv. Huia) - *Poa pratensis* L. (cv. Sobra);
4. *Trifolium pratense* L. (cv. Altaswede) - *Phleum pratense* L. (cv. Erecta);
5. *Medicago sativa* L. (local population) - *Dactylis glomerata* L. (cv. Loke)
6. *Trifolium pratense* L. (cv. Altaswede) - *Festuca pratensis* L. (cv. Laura).

The experiment was based on the block method, in 4 replicates with a plot size of 5 m^2. Sowing was done manually, in a scattered way, with a sowing rate consistent with that of the species in a pure crop at a ratio of 50: 50. Cultivation of the mixtures was without fertilizing under nonirrigated conditions. The grass stands were mowed at the beginning of the blossoming phase of legumes and tasseling/ear formation for the grasses. In total, six regrowths were harvested during the experimental period.

The yield of crude protein (CP, kg/da) and the yield of feed units (feed unit for milk - FUM and feed unit for growth - FUG) were established by the yield of dry matter (kg/da) and the content of the indicators in 1 kg of dry matter.

Statistical processing of the obtained results was performed with ANOVA, LSD test for statistical significance of differences, standard deviation and coefficient according to the variational statistical method (Lidanski, 1988).

RESULTS AND DISCUSSIONS

Competition among species is an essential factor for the optimal performance of fodder crops in the grass stand. In this case, the mixture of *Lotus corniculatus* L. with *Festuca rubra* L. gives a clear idea of a good balance between the components, which is a prerequisite for the formation of a highly productive fodder mass (Figure 1).

Regarding the cenotic activity of the components in the *Medicago sativa* L.-*Dactylis glomerata* L. mixture, the grass crop prevailed in the total yield by more than 75% compared to the legume, which affected the nutritional value of the grass mass.

According to Bozhanska (2017), the fodder from the mixture has a low crude protein content, a high concentration of fibrous components in the cell walls and the lowest *in vitro* digestibility of dry matter (from 0.3% to 13%) compared to the digestibility of the remaining mixtures included in the experiment.

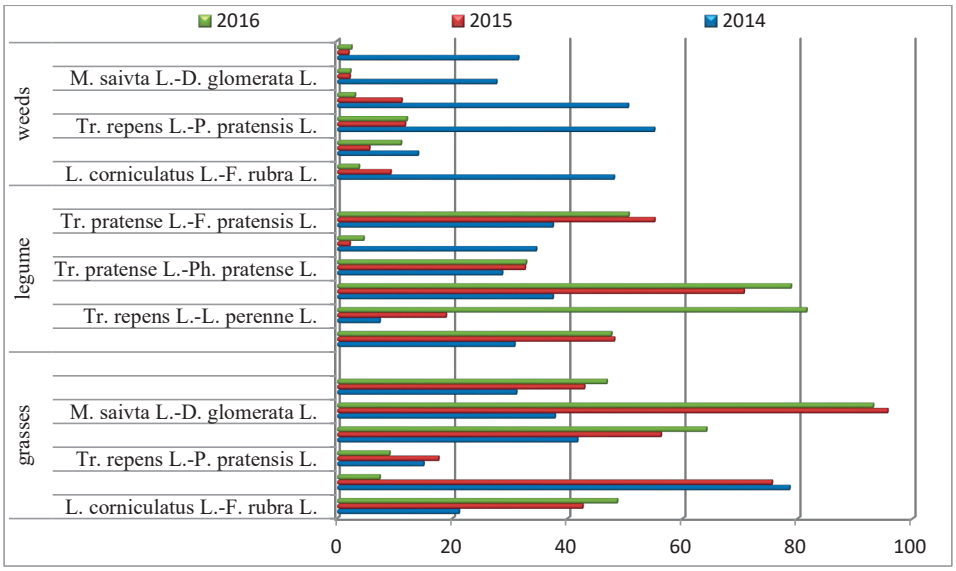

Figure 1. Botanical composition (percentage of components in the grass stand) of perennial grass-legume mixtures, over the years (%)

Lotus corniculatus L. and *Trifolium repens* L. are legume crops of high fodder significance for the Central Balkan Mountain region (Mihovski and Goranova, 2006; Churkova, 2019), whereas *Festuca rubra* L. and *Poa pratensis* L. are the main structure-determining grass components in the composition of mountain grass stands (Mitev and Goranova, 2008; Mitev et al., 2010).

The share of fodder crops in the composition of perennial mixtures changes with increasing the age of the grass stand, which affects the quality and biochemical characteristics of fodder.

The mixed grass stands of *Trifolium repens* L. and *Trifolium pratense* L. with *Poa pratensis* L. and *Festuca pratensis* L., respectively, were the only ones in which the legume component predominated in all regrowths during the three-year experimental period. The ratio of legume and grass crops in the mixed grass stands of *Trifolium repens* L. and *Poa pratensis* L. was 62.3: 13.9%, and in those of *Trifolium pratense* L. and *Festuca pratensis* L., respectively - 47.7: 40.3%.

The development and composition of perennial grasses and grass mixtures is related to a complex of conditions (soil, climate, agrotechnical, biological, etc.) that have an impact on their bioproductivity and quality characteristics. Grass-legume mixtures are preferred over pure grass stands throughout the world because they often increase the total yields of fodder and protein and offer balanced nutrition (Albayrak et al., 2011).

In the year of sowing, crude protein yield in the mixed two-component grass stands ranged from 86.72 kg/da (*Trifolium pratense* L. - *Festuca pratensis* L.) to 114.13 kg/da (*Lotus corniculatus* L. -*Festuca rubra* L.) (Table 1). The excess in the values of the indicator compared to the control is from 1.75% to 18.7%. In the second vegetation (2015), when legume crops reached their maximum development, mixtures of *Trifolium pratense* L., as well as those of *Trifolium repens* L. with *Poa pratensis* L., and *Lotus corniculatus* L. with *Festuca rubra* L., registered from 1.2% to 20.3% higher yield of crude protein compared to the average value of the indicator (71.65 kg/da). In the third vegetation, the highest yield of crude protein was found in the mixed grass stands of *Trifolium repens* L. with *Poa pratensis* L. (139.47 kg/da) followed by *Lotus corniculatus* L. with *Festuca rubra* L. (118.11 kg/da), *Trifolium repens* L. with *Lolium perenne* L. (117.07 kg/da) and *Trifolium pratense* L. with *Festuca pratensis* L. (115.02 kg/da). The excess in the values of the indicator compared to the control is from 4.8% to 26.8%.

Table 1. Yield of crude protein (CP) in the biomass of perennial two-component mixtures, over the years and average for the period 2014-2016 (kg/da)

Variants	2014	2015	2016	2014-2016
Lotus corniculatus L.-*Festuca rubra* L.	114.13	72.53	118.11	102.97
Trifolium repens L.-*Lolium perenne* L.	97.83	56.20	117.07	88.71
Trifolium repens L.-*Poa pratensis* L.	94.75	82.48	139.47	105.30
Trifolium pratense L.-*Phleum pratense* L.	93.77	80.00	95.70	90.14
Medicago saivta L.-*Dactylis glomerata* L.	89.68	52.50	74.54	72.45
Trifolium pratense L.-*Festuca pratensis* L.	86.72	86.21	115.02	95.94
Average	***96.15***	***71.65***	***109.99***	***92.59***
Min	**86.72**	**52.50**	**74.54**	**72.45**
Max	***114.13***	***86.21***	***139.47***	***105.30***
CV	***10.0***	***19.8***	***20.2***	***12.8***
SD	***9.64***	***14.18***	***22.23***	***11.89***
LSD$_{0.05}$				***17.6***

The highest average yield of crude protein, for the experimental period, was found in the mixtures - *Trifolium repens* L. - *Poa pratensis* L. (105.30 kg/da), *Lotus corniculatus* L. - *Festuca rubra* L. (102.97 kg/da) and *Trifolium pratense* L. - *Festuca pratensis* L. (95.94 kg/da) (in the conditions of the Central Balkan Mountain).

The excess in the values of the indicator compared to the control is from 13.7%, 11.2% and 3.6%. The lowest yield values for crude protein in the second (52.50 kg/da) and third (74.54 kg/da) experimental year, as well as the average for the period (72.45 kg/da) was registered in the mixture of *Medicago sativa* L. with *Dactylis glomerata* L. The values of the trait are lower by 20.8% compared to the average for a three-year period (92.59 kg/da) and by 31.2% compared to the highest average value of the indicator.

A proven difference in the yield of crude protein ($P < 0.05$) was found between the *Medicago saivta* L. - *Dactylis glomerata* L. mixture and all the other mixed grasses included in the study (except for the *Trifolium repens* L. - *Lolium perenne* L. mixture).

There is also a proven difference in the yield of crude protein between the two-component mixtures of *Trifolium repens* L.

The amount of the trait in the grass stand with *Poa pratensis* L. (105.30 kg/da) is higher than that in the mixtures with *Lolium perenne* L. (88.71 kg/da) and is proportionally dependent on the higher share of the bean component in the volume of the grass mass.

The mixtures of *Trifolium pratense* L. have close average values of the indicator for the study period. With more distinct differences in the yield of crude protein are the variants of the legume crop in the third growing season. The crude protein content of the biomass of *Trifolium pratense* L. -*Festuca pratensis* L. mixture was 20.2% higher compared to that of *Trifolium pratense* L. - *Phleum pratense* L. (95.70 kg/da).

The soil profile in the experimental area does not meet the requirements of plants of the *Medicago saivta* L species. This is one of the main reasons for the unsustainable participation of the leguminous component (a decrease in the percentage share of the crop was registered by year) in the mixed with *Dactylis glomerata* L. and the formation of forage mass with the lowest values of crude protein.

The energy value of the fodder mass, expressed in terms of feed units for milk and growth, is the main criterion for modern assessment of fodder quality (Todorov, 1997).

In the first and second experimental years, as well as on average over the study period, the fodder mixture of *Trifolium pratense* L. with *Phleum pratense* L. had the highest yield of feed units for milk and growth (Table 2). Values of the indicators exceeded the averages respectively by:

- 8.27% (FUM) and 8.59% (FUG) - in the first experimental year;
- 24.3% (FUM) and 25.9% (FUG) - in the second experimental year;
- 9.5% (FUM) and 11.0% (FUG) - on average for the period 2020-2022.

For the study period, the yield of fodder units in the fodder mass of the two-component mixtures - *Lotus corniculatus* L.- *Festuca rubra* L., *Trifolium pratense* L.- *Festuca pratensis* L. and *Trifolium repens* L.- *Poa*

pratensis L. also exceeded the average values of the indicator by 8.7%, 1.9% and 0.7% (for FUM) and 8.1%, 1.2% and 0.2% (for FUG), respectively.

Table 2. Yield of feed units (FUM and FUG) in the biomass of perennial two-component mixtures, over the years and average for the period 2014-2016 (MJ/da)

Variants	2014		2015		2016		2014-2016	
	FUM	FUG	FUM	FUG	FUM	FUG	FUM	FUG
L. corniculatus L.-*F. rubra* L.	469.80	428.30	402.90	368.90	673.20	613.80	517.10	472.10
Tr. repens L.-*L. perenne* L.	409.40	373.30	363.10	331.50	528.90	493.10	430.00	399.30
Tr. repens L.-*P. pratensis* L.	456.70	415.80	384.70	358.40	586.20	525.00	479.20	437.60
Tr. pratense L.-*Ph. pratense* L.	476.00	435.20	519.70	483.10	576.90	529.50	521.20	484.50
M. saivta L.-*D. glomerata* L.	413.80	376.80	353.20	316.30	500.40	455.60	422.50	384.60
Tr. pratense L.-*F. pratensis* L.	412.10	375.20	485.90	444.20	563.90	514.10	484.70	441.90
Average	***439.63***	***400.77***	***418.25***	***383.73***	***571.58***	***521.85***	***475.78***	***436.67***
Min	***409.40***	***373.30***	***353.20***	***316.30***	***500.40***	***455.60***	***422.50***	***384.60***
Max	***476.00***	***435.20***	***519.70***	***483.10***	***673.20***	***613.80***	***521.20***	***484.50***
CV	***7.1***	***7.2***	***16.4***	***17.2***	***10.4***	***10.1***	***8.8***	***9.0***
SD	***31.19***	***28.82***	***68.56***	***65.83***	***59.19***	***52.53***	***41.94***	***39.19***
$LSD_{0.05}$							***57.5***	***52.8***

In the first experimental year, higher content of fodder units were registred in comparison with the average values of the indicators in the dry matter of the two-component mixtures of *Lotus corniculatus* L. - *Festuca rubra* L. (469.80 - FUM and 428.30 - FUG) and *Trifolium repens* L. -*Poa pratensis* L. (456.70 - FUM and 415.80 - FUG). The careful and purposeful approach among them is a prerequisite for better distribution of components in plant communities and production of feed biomass with high economic value (Vîntu et al., 2011; Fattahi and Ildoromi, 2011; Zziwa et al., 2012; Iliev et al., 2022). In the second experimental year, an excess in the values of the studied indicators was registered in the mixed crops of *Trifolium pratense* L. with *Festuca pratensis* L. (485.90 - FUM and 444.20 - FUG), whereas in the third experimental year, higher values were found in the legume-grass grass stands of *Trifolium repens* L. with *Poa pratensis* L. (586.20 - FUM and 525.00 - FUG) and *Trifolium pratense* L. with *Phleum pratense* L. (576.90 - FUM and 529.50 - FUG).

A proven difference ($P < 0.05$) was found in the yield of feed unit for milk and growth between the fodder mass of *Trifolium pratense* L. - *Phleum pratense* L. and that of the two-component mixtures - *Medicago saivta* L. - *Dactylis glomerata* L. (with 23.4% for FUM and 26.0% for FUG) and *Trifolium repens* L. - *Lolium perenne* L. (with 21.2% for FUM and 21.3% for FUG).

Species diversity in the composition of grass crops affects the quality and nutritional value of the fodder mass (Belesky et al., 1999; Sanderson et al., 2005; Foster et al., 2019). The percentage share of legume and grass components in grass mixtures is a significant factor for the formation of highly productive biomass to meet the needs of the livestock sector (Vasylenko et al., 2020).

In the present study, the the ratio of species (legume and grass meadow crops) in the mixed grass stands accounted for a significant share of the factorial variance in the studied traits (Table 3).

Table 3. Degree and significance of the factorial influences on the yield of CP, FUM and FUG in mixed grass stands of legume and grass meadow crops

Sources of variation / Traits	Year and age of grass stand		Type of grass stand	
	η^2 (%)	P	η^2 (%)	P
CP Yield	24.38	ns	53.4	P <0.01
Yield of FUM	20.15	ns	64.3	P <0.001
Yield of FUG	21.16	ns	63.2	P <0.001

Significance of differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$; ns - not significant; η^2 - correlation ratio of factorial to total variance

The factor - type of grass stand had a significant impact ($P < 0.05$ - $P < 0.001$) on the values of the indicators: yield of crude protein (η^2 - 53.4%), yield of feed units for milk (η^2 - 64.3%) and feed units for growth (η^2 - 63.2%).

The analyzed data show also that the agro-ecological characteristics (temperature and precipitation in the area of the experiment) and the age of the examined grass stands did not significantly affect the values of the indicators - CP, FUM and FUG.

CONCLUSIONS

Crude protein yield in the investigated mixed grass stands exceeded the average value of the indicator (92.59 kg/da) by 3.6% (*Trifolium pratense* L. - *Festuca pratensis* L.) to 13.7% (*Trifolium repens* L. - *Poa pratensis* L.). The indicated variants are the only ones in which the legume component prevailed in the regrowth biomass throughout the experimental period in a ratio of legume and grass crops of 47.7:40.3% respectively (for *Trifolium pratense* L. - *Festuca pratensis* L.) and 62.3:13.9% (for *Trifolium repens* L. - *Poa pratensis* L.). A proven difference ($P < 0.05$) in crude protein yield was found only between *Trifolium repens* L. - *Poa pratensis* L. and *Medicago sativa* L. - *Dactylis glomerata* L. mixtures.

The fodder mass of the mixture *Trifolium pratense* L. with *Phleum pratense* L. had the highest yield of feed units for milk and growth. The values of both indicators exceeded the average (FUM - 475.78 MJ/da and FUG - 436.67 MJ/da) respectively with 9.5% (FUM) and 11.0% (FUG). There is a proven difference ($P < 0.05$) in the yield of feed units for milk and growth between the fodder mass of *Trifolium pratense* L.-*Phleum pratense* L. (FUM - 521.20 MJ/da and FUG - 484.50 MJ/da) and that of the two-component mixtures - *Medicago sativa* L. - *Dactylis glomerata* L. (FUM - 422.50 MJ/da and FUG - 384.60 MJ/da) and *Trifolium repens* L.-*Lolium perenne* L. (FUM - 430.00 MJ/da and FUG - 399.30 MJ/da) .

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