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Forage quality characteristics of meadow legume crops grown under mountain conditions

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Abstract

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During the period 2020-2022, the following types of meadow legume crops were tested on light gray pseudopodzolic soil in the experimental field of Research Institute of Mountain Stockbreeding and Agriculture-Troyan under mountain conditions: *Lotus corniculatus* L.*, Trifolium hibridum* L.*, Medicago sativa* L.*, Trifolium pratense* L.*, Trifolium repens* L. The aim of the study was to determine their basic chemical composition. It was found that the grass stand type had a greater effect on the content of calcium, crude protein, crude fiber, and nitrogen-free extractable substances and to a lesser extent on crude fat and phosphorus. White clover combined the highest percentage of crude protein and the lowest in crude fiber, whose content was preserved over the years. Grass stands with bird's-foot-trefoil and hybrid clover increased their protein content in the course of years. The least share of crude fiber was registered in white and hybrid clover (31.68% and 33.85%), whereas the most in alfalfa (44.23%). White clover is the most suitable type of legumes for mountain conditions. Moreover, it registered the highest crude protein content, the lowest crude fiber content and had the highest nitrogen-free extractable substances and phosphorus content.

Keywords: Legume crops; chemical composition; statistical processing

Introduction

For the conditions of sustainable agriculture, legumes are becoming increasingly popular (Wezel & Jauneau, 2011; Bozhanska et al., 2016). Thanks to their nitrogen-fixing ability, they are an alternative to industrial nitrogen fertilizers and a way to protect the environment (Peeters et al., 2006).

The amount of fixed nitrogen depends on the type of legume crop and its symbiosis with bacteria from the genera *Rhizobium* and *Bradyrhizobium* (Donkova et al., 2014). Alfalfa, clover and bird's-foot-trefoil accumulate 180, 170 and 92 kg/ha^{-1} N per year in the soil, respectively, and exhibit good adaptability to soil and climate conditions, altitudes and other environmental conditions (Frame, 2005).

Legumes are a source of high-quality fodder and contribute to reducing greenhouse gas emissions. Introduced into modern cropping systems, they increase crop diversity and reduce the use of external inputs. They release high-quality organic matter into the soil and facilitate soil nutrient circulation and water retention. Based on these multiple functions, legumes have a high potential for conservation agriculture (Stagnari et al., 2017).

Fodder quality depends on the basic chemical composition of legume crops, and it is largely determined by the cultivation technology applied, species and cultivar characteristics, leaf-to-stem ratio, growth phase, soil agents, climate, harvest phase, diseases and pests (Arzani et al., 2001). The highest crude protein is found during the early phases of plant development and decreases with advancing plant age (Kirilov, 2010). The greater the degree of leafiness of the plants, the higher the protein content. Crude fiber depends on the intensity of stem growth and plant height (Kirilov, 2016).

Alfalfa, bird's-foot-trefoil, sainfoin, white and red clover are widely used legume grass fodder crops (Vasileva & Ilieva,

2009; Churkova, 2018). The yield and crude protein content of dry matter of herbaceous species are important parameters in determining their fodder value. The quality of the dry matter of herbaceous species depends on the content of macro and microelements in its composition. Therefore, the ratio of calcium and phosphorus, as well as the numerical values of nitrogen-free extractable (NFE) substances, are important indicators affecting the nutritional value of legume hay.

Sainfoin (*Onobrychis* Paertn.) contains less crude protein and more nitrogen-free extractable substances than alfalfa, and therefore its general nutritional value is not inferior (Kyuchukova & Ilieva, 2008).

The average value of crude protein in some bird's-foot-trefoil cultivars grown under mountain conditions was 129.8 $g.kg^{-1}$, crude fiber was from 225.8 $g.kg^{-1}$ to 288.1$g.kg^{-1}$, and the average value of ash was 81.3 $g.kg^{-1}$ (Churkova, 2013; Churkova & Churkova, 2022).

White clover (*Trifolium repens* L.) is one of the main crops in organic and sustainable agriculture. Its high fodder quality, excellent animal uptake and nitrogen economy characterize it as the most efficient meadow legume crop (Frame, 2000; Stypinsky & Redzinska, 2003).

Alfalfa (*Medicago sativa* L.) grass mass consists of a protein-rich, highly digestible leaf fraction and a less digestible carbohydrate-rich stem fraction (Wilman & Altimimi, 1984; Kertikova et al., 2014).

In the strategic guidelines for the development of fodder production and meadow farming, one of the goals is the implementation of suitable meadow legume crops with highly effective strains of tubercle bacteria, which will lead to the improvement of the technology for creating grass stands and to the realization of production from them with a high economic, applied and ecological effect.

The purpose of the present research is to study the main chemical composition of meadow legume crops grown under mountain conditions.

Material and Methods

The experiment was conducted during the period 2020-2022 on the territory of the experimental field of Research Institute of Mountain Stockbreeding and Agriculture-Troyan, on light gray pseudopodzolic soil. The following types of meadow legume crops were tested: *Lotus corniculatus* L.*, Trifolium hibridum* L.*, Medicago sativa* L.*, Trifolium pratense* L.*, Trifolium repens* L. A generally accepted technology for growing meadow legume crops for fodder was applied. The sowing rate for herbaceous species was as follows: for bird's-foot-trefoil and white hybrid clover was 12 t/ha, and for alfalfa and red clover was 25 t/ha. The sowing of the grass stand was done manually, in a scattered manner with a different sowing rate according to the type of crop. The experiment was sown according to the block method in 4 replications with a harvest plot size of 5 m^2. No fertilizing was applied during the research experiment.

In the first year, one regrowth was harvested, and in the remaining two years, two regrowths were harvested in the bud-formation period-beginning of blossoming phase of the legume crops.

The samples for the chemical analysis of the fodder were taken in the bud-formation period – the beginning of blossoming of the legumes by regrowths, and the results presented are of the data obtained by year and averaged over the experimental period. Plant materials were dried under natural conditions. Immediately before grinding, the samples were placed in a laboratory dryer at a temperature of 60°C, to facilitate grinding. Grinding was conducted in a laboratory mill to a particle size of 1.0 mm. The main chemical composition was analyzed according to the Weende analysis of the dried and ground grass biomass of each variant including: Crude protein (CP, $g\ kg^{-1}$) according to Kjeldahl (according to BDS/ISO-5983); Crude fiber (CFr, $g\ kg^{-1}$); Crude fats (CF, $g\ kg^{-1}$) (according to BDS/ISO-6492) – by extraction in a *Soxhlet* type extractor; Ash ($g\ kg^{-1}$) – (according to BDS/ISO-5984) decomposition of organic matter by gradually burning the sample in a muffle furnace at 550°C; Dry matter (DM, $g\ kg^{-1}$) – empirically calculated from % of moisture; NFE = 100 – (CP, % + CFr, % + CF, % + Ash, % + Moisture, %) converted to $g\ kg^{-1}$; Calcium (Ca, $g\ kg^{-1}$) – according to Stotz (complexometric) and Phosphorus (P, $g\ kg^{-1}$) – with a vanadate-molybdate reagent according to the Gerike and Kurmis method – spectrophotometer (Agilent 8453 UV – visible Spectroscopy System), measuring in the 425 ηm region.

Average (×), minimum (min) and maximum (max) limits of chemical analysis data were calculated (Lidanski, 1988). The degree of variation of the parameters was determined by the variation coefficient (VC) according to the Mamaev scheme: up to 7% – very low; 7.1 to 12.0% – low; 12.1 to 20.0% – moderate; 20.1 – 40.0% – high; above 40.0% – very high. The variation coefficient measures variability relative to the mean and is found by dividing the standard deviation by the mean. It is a statistical metric that shows the dispersion of data points that are around the mean.

Results and Discussion

Table 1 presents the main chemical composition of grass stands with meadow legume crops in the first experimental year (2020). Crude protein has the maximum value in the fodder mass of *Trifolium repens* L. (17.12%). Red clover

recorded the lowest protein content (13.24%). The difference in the protein content of the tested legume grasses is small, which also determines the low variation coefficient (CV = 10.26%) and the low value of the standard deviation (SD = 1.58). Crude fiber values were significantly high and ranged from 40.05% in the fodder mass of white clover to 48.32% in red clover. As crude fiber increases, protein content decreases. This is evidenced by the lowest value of crude protein (13.24%) and the highest crude fiber (48.32%) of the red clover fodder mass, and the highest crude protein content (17.12%) registered the lowest percentage of raw fibers (40.05%). The mean values of crude protein and crude fiber were 15.37% and 44.54%, respectively. Both in terms of crude protein and crude fiber, no significant difference in values was found for the legume species tested. This also determines the very low coefficient of variation of raw fibers (CV = 6.99). The mineral content ranged from 6.09% to 6.92% with an average value of 6.39% and a very low degree of variability according to the coefficient of variation (CV = 6.18%). NFE has the highest value in bird's-foot-trefoil (27.15%) and the lowest in hybrid clover (17.19%) with an average value (21.52%). Crude fat ranges from 2.43 to 4.12% with maximum values in *Trifolium pratense* L.

The difference in the crude fat content of the fodder of the legumes is substantial, which is evident from the high degree of variability according to the coefficient of variation (CV = 22.37%). The type of legumes has the greatest impact on the content of calcium and phosphorus in the dry matter of the fodder. Calcium ranges from 0.84% to 1.82%, and phosphorus from 0.01% to 0.16%.

The type of fodder from the different legume crops has the greatest impact on the content of the macronutrients, such as calcium and phosphorus, which is evident from the highest values of the coefficients of variation, respectively CV = 31.51% for calcium and CV = 99.41% for phosphorus, with average values 1.33% and 0.07%. The lower content of crude protein and the higher content of crude fiber are related to the morphological composition of the grass stand, which shows that a greater part of the above-ground mass is represented by stems, and they contain more fiber.

In the second experimental year (Table 2), the rate of plant growth and development affects the values of the indicators of the chemical composition of the grass stand. Crude protein increased significantly in bird's-foot-trefoil and red clover, 17.07% and 17.23%, respectively, with values of 14.28% and 13.24% in the first year. This is due to the high leafiness of the plants of both species, and according to Kirilov (2016), the greater the proportion of leaves in the grass

Table 1. Main chemical composition (% of dry matter) of meadow legume crops in the first experimental year (2020)

Legumes	Crude protein	Crude fiber	Crude fat	Ash	NFE	Ca	P
Lotus corniculatus L.	14.28	45.8	2.43	6.92	27.15	0.84	0.03
Trifolium hibridum L.	16.33	45.4	2.87	6.09	17.19	1.13	0.12
Medicago sativa L.	15.86	43.12	3.94	6.12	18.03	1.72	0.02
Trifolium pratense L.	13.24	48.32	4.12	6.72	22.14	1.15	0.01
Trifolium repens L.	17.12	40.05	2.96	6.12	23.07	1.82	0.16
Average (×)	15.37	44.54	3.26	6.39	21.52	1.33	0.07
SD	1.58	3.11	0.73	0.40	4.04	0.42	0.07
VC	10.26	6.99	22.37	6.18	18.79	31.51	99.41
min	13.24	40.05	2.43	6.09	17.19	0.84	0.01
max	17.12	48.32	4.12	6.92	27.15	1.82	0.16

Table 2. Main chemical composition (% of dry matter) of meadow legume crops in the second experimental year (2021)

Legumes	Crude protein	Crude fiber	Crude fat	Ash	NFE	Ca	P
Lotus corniculatus L.	17.07	25.75	4.56	11.12	32.13	1.02	0.01
Trifolium hibridum L.	15.51	28.74	2.09	6.48	37.01	1.02	0.01
Medicago sativa L.	13.08	50.29	1.87	9.26	16.22	2.34	0.02
Trifolium pratense L.	17.23	44.70	2.10	7.31	19.03	2.12	0.01
Trifolium repens L.	17.70	32.51	2.06	6.20	31.31	1.56	0.01
Average (×)	16.12	36.40	2.54	8.07	27.14	1.61	0.01
SD	1.89	10.60	1.14	2.08	9.01	0.61	0.00
VC	11.71	29.11	44.77	25.77	33.20	3.78	3.71
min	13.08	25.75	1.87	6.20	16.22	1.02	0.01
max	17.7	50.29	4.56	11.12	37.01	2.34	0.02

mass, the higher the protein content in the fodder. The average protein value of the tested species (×) was 16.12% with a coefficient of variation (VC) of 11.71% and a standard deviation (SD) of 1.89. The low protein of alfalfa is impressive, as evidenced by the higher relative proportion of stems and the higher values of their stem aspect, confirmed by the research of Arzani et al. (2001). Crude fiber in bird's-foot-trefoil, hybrid and red clover decreased compared to the first year.

The amount of crude fiber was significantly higher depending on the type of legume component compared to the first year. The variation coefficient is a proof, which increased from 6.99% in the first experimental year to 29.11% in the second year. The difference from the minimum and maximum value is twice, 25.75% and 50.29%, respectively. The amount of mineral substances varies widely (from 6.20% to 11.12%), which proves the high degree of variability according to the coefficient of variation (CV = 25.77%). The nitrogen-free extractable substances in the fodder of bird's-foot-trefoil, hybrid and white clover were twice as high as those of alfalfa and red clover. Their average value is 27.14% and the variation coefficient (CV) 33.20% with a very high degree of variability. Calcium varies from 1.02% to 2.34%, with the maximum amount for alfalfa (*Medicago sativa* L.). The phosphorus content is in the range of 1.01% to 1.02% with a low coefficient of variation (CV = 3.71%). Alfalfa registered the most phosphorus, which proves the highest content of macronutrients in the fodder of this legume species.

In the third experimental year (Table 3), white clover preserved the highest content of crude protein in the dry matter composition, respectively 17.60%, and its amount was approximately the same in the three experimental years. The largest leafiness of white clover also determines the highest protein content. The protein percentage in the fodder of red clover is lower (12.53%), because of its high presence in the grass stands in the first and second year of its development and with the increase in the height and the relative share of the stems in the grass stands.

The average protein value of the tested legumes was 14.79% and was the lowest compared to the previous two years. Alfalfa and red clover have the lowest crude protein (11.05% and 12.53%), which proves that the advancing age of the grass stand does not affect the protein content of white clover and alfalfa, but it does in bird's-foot-trefoil and hybrid clover. This confirms the results obtained by Kirilov (2010), that as the leafiness of the plants increases, the protein content in the fodder also increases. Crude fibers in the third year have the lowest values, with the lowest amount in white clover (22.48%) and the highest in red clover (39.28%). Crude fat ranges from 1.64% to 3.33% with an average value of 2.17%. The highest amount of mineral substances is found in white clover (8.84%) and the least in red clover (7.15%), and the average value is 8.02%. Calcium in the fodder mass of bird's-foot-trefoil, alfalfa, white and red clover has similar values, with an average value of 2.10%. Phosphorus varied from 1.29% to 1.336%, and the difference in its content by species was insignificant, which was accounted for by the very low value of the coefficient of variation (CV = 1.42%). According to the values of the variation coefficient, the degree of variability of the parameters, such as crude fat, crude fiber, crude ash is the highest, and phosphorus, calcium, NFE substances and ash are the lowest.

On average for the period 2020-2022 (Table 4), the crude protein content in the dry fodder mass of white clover, hybrid clover and bird's-foot-trefoil is 16.87%, 15.92% and 15.01%, which proves that these species are suitable for growing in mountain conditions according to this indicator. In the case of alfalfa and red clover, no effect was registered on the crude protein indicator, which is confirmed by the obtained data close in value, respectively (12.92% and 13.78%).

The percentage difference between the maximum mean values of the tested legume crops was different, indicating that the type of legumes affects the fodder protein. The fodder mass of alfalfa (44.23%) has the highest percentage of crude fiber followed by that of red clover (41.09%).

Table 3. Main chemical composition (% of dry matter) of meadow legume crops in the third experimental year (2022)

Legumes	Crude protein	Crude fiber	Crude fat	Ash	NFE	Ca	P
Lotus corniculatus L.	15.27	32.43	1.64	7.59	43.06	2.14	1.325
Trifolium hibridum L.	17.49	27.42	2.02	8.24	44.83	1.92	1.316
Medicago sativa L.	11.05	39.28	1.94	8.28	39.46	2.14	1.336
Trifolium pratense L.	12.53	32.77	1.94	7.15	45.61	2.15	1.286
Trifolium repens L.	17.60	22.48	3.33	8.84	47.74	2.16	1.313
Average (×)	14.79	30.88	2.17	8.02	44.14	2.10	1.32
SD	2.94	6.31	0.66	0.66	3.11	0.10	0.02
VC	19.86	20.42	30.47	8.20	7.04	4.86	1.42
min	11.05	22.48	1.64	7.15	39.46	1.92	1.29
max	17.60	39.28	3.33	8.84	47.74	2.16	1.336

Table 4. Main chemical composition (% of dry matter) of meadow legume crops averaged over the study period (2020–2022)

Legumes	Crude protein	Crude fiber	Crude fat	Ash	NFE	Ca	P
Lotus corniculatus L.	15.01	35.50	2.88	8.54	34.11	1.33	0.455
Trifolium hibridum L.	15.92	33.85	2.33	6.94	33.01	1.36	0.482
Medicago sativa L.	12.92	44.23	2.58	7.89	24.57	2.07	0.459
Trifolium pratense L.	13.78	41.09	2.72	7.06	28.93	1.81	0.435
Trifolium repens L.	16.87	31.68	2.78	7.05	34.04	1.85	0.494
Average (×)	14.90	37.27	2.66	7.50	30.93	1.68	0.47
SD	1.59	1.59	0.21	0.70	4.14	0.32	0.02
VC	10.65	14.01	8.04	9.31	13.39	19.22	5.01
min	12.92	31.68	2.33	6.94	24.57	1.33	0.44
max	16.87	44.23	2.88	8.54	34.11	2.07	0.49

The tendency to increase the crude protein content when reducing protein is very pronounced. This is evident from the variation coefficient values, according to which, in relation to crude protein (VC = 10.65%), the degree of variability is low, and according to crude fiber, it is average (VC = 14.01%). The standard deviation has relatively low values for both indicators, 1.59 and 1.59, respectively. The type of grass stand has the slightest impact on crude fat according to the variation coefficient (VC = 8.04%). The reported average value is 2.66% with a minimum value (2.33%) and a maximum value (2.88%). The ash content of the dry matter ranged from 6.94% to 8.54% with an average value of 7.50%. The degree of variability is low 9.31%, which proves that the type of grass stand does not affect this indicator. Nitrogen-free extractable substances ranged from 24.57% to 34.11% with an average degree of variability according to the coefficient of variation (13.39%). Calcium is most affected by the type of grass stand, which is evident from its highest coefficient of variation (19.22%), whereas it is the leatest for phosphorus (5.01%). The content of calcium varies from 1.33% to 2.07%, and of phosphorus from 0.44% to 0.49%.

Conclusions

The results of the comparative test of different types of meadow legume crops under the conditions of the Central Balkan Mountain showed that the choice of species has a significant impact on the quality of the fodder when grown as a pure crop. It was established that the morphological composition of meadow legume crops and the conditions in the area of cultivation, influence the indicators of the main chemical composition of the grass stands and are determining factors for the nutritional value of the fodder mass. The highest content of crude protein and the lowest of crude fiber is in the biomass of *Trifolium repens* L. The type of grass stands in the studied meadow legume crops affects to a greater extent the content of calcium, crude protein, crude fiber, and NFE substances and to a lesser extent on crude fats, calcium and phosphorus. Grass stand age affected crude protein in white clover, which was relatively constant across years, and in bird's-foot-trefoil and hybrid clover it increased with years of the experimental period. The least share of crude fiber was registered in white and hybrid clover (31.68% and 33.85%), whereas the most in alfalfa (44.23%). White clover is the most suitable type of legumes for mountain conditions. Moreover, it registered the highest crude protein content, the lowest crude fiber content and had the highest NFE substances and phosphorus content.

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