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Chemical composition of bird's foot trefoil cultivars grown in mountain conditions

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Abstract

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Chemical analysis on fodder of bird's foot trefoil cultivars was conducted: Targovishte 1, Alvena (Italy), Lotanova (Italy), Frilo (Italy), Polom (Slovakia), Bonnie (France), Bull (Canada), grown in mountain conditions in the period 2016-2019. The indicators, such as crude proteins, crude fats, crude fiber, crude ash were observed and the correlation dependences between them were calculated, such as yield and morphological composition of the grassland. After their statistical processing and comparative evaluation, the variability of the indicators over the years was established.

Bull cultivar stood out as the most promising in terms of the amount of crude protein for use in selection programs and for implementation in production in mountain conditions, and Bonnie cultivar in terms of crude fiber and the amount of minerals. Fats registered maximum values for Frilo cultivar. The indicators of crude protein, crude fiber and crude ash showed a very low degree of variability, while for the indicator of fat it was low.

The established strong positive correlation between the content of crude protein and the percentage of bird's foot trefoil in grassland (r = 0.8516) on the one hand, and the content of crude fiber and crude protein (r = 7851) on the other hand, showed the interdependence between the indicators. This determines the benefits of growing these cultivars in mountain conditions and their significance for animal feeding.

Keywords: bird's foot trefoil; cultivars; chemical composition; correlation coefficients

Introduction

Grass fodder is essential for ruminant feeding in terms of energy, protein and mineral supply (Karabulut et al., 2007; Bozhanska et al., 2015). Bird's foot trefoil is a widespread legume grass species in Bulgaria and abroad, due to the high yield of fodder, good tolerance of acidic and poorly fertile soils (Maroso et al., 2009; Bozhanska, 2020) and excellent nutritional value (Heuzé et al., 2016). It is a high-quality, nitrogen-fixing perennial legume that increases the efficiency of nitrogen and water use in artificial grasslands (Macadam & Griggs, 2013). The characteristics of morphological and qualitative indicators of individual cultivars and populations of bird's foot trefoil allow the use of genetic material for selection purposes (Stojanovic et al., 2007). The nutritional value of fodder is determined by the dry matter digestibility (Kaplan et al., 2009). The relative fodder value is a widely accepted fodder quality index and is determined by the content of acidic (ADF) and neutral (NDF) detergent fibers (Caddel, 2005).

The quality of the fodder mass of legumes depends on the time of mowing (Charalampos et al., 2019). The crude protein of bird's foot trefoil harvested during the bud-formation period according to Abdushaeva (2020) was 14.4%, and (Ramirez-Restrepo et al., 2006) determined an amount of 10.56 to 21.93%, which decreased with increasing maturity of plants. Peiretti et al. (2016) presented the following chemical composition of *Lotus corniculatus*: 88 ash, 84

monosaccharides, 44 starch, 45 pectin, 485 NDF, 103 lignin, 27 N g/kg DM. Fodder shortages and poor feed quality during the dry season are the main production constraints in the livestock sector. Ideal pasture and fodder species should be highly profitable and tasty and contain adequate levels of well-digested nutrients to meet animal requirements (Kisitu, 2010). The nutritional value of plants has been used to guide ecosystem management strategies, including the selection of fodder species (Delaby & Peyraud, 2009). The fodder quality determines the supply of nutrients needed by an animal, both quantitatively and qualitatively, to support the desired type of production. When the chemical composition and fodder digestibility are known, it is possible to calculate its energy content using appropriate regression equations (Mlay et al., 2006). This determined the need to analyze the nutritional value of different cultivars of bird's foot trefoil grown under the specific conditions of a given region.

The aim of the present study was to study bird's-foot-trefoil cultivars in terms of crude protein, crude fiber, fat and crude ash with a view to include them in selection programs and to establish their suitability to be introduced in production.

Material and Methods

The study was conducted on light grey pseudo podzolic soil during 2016-2019. Soil preparation was according to the adopted technology of the IMSA in Troyan. The trial was laid out by the block method with 4 replications and size of harvest plot of 1 m^2. Objects of study were the cultivars originating from: Targovishte 1, 2 Alvena (Italy), 3. Lotanova (Italy), 4. Frilo (Italy), 5. Polom (Slovakia), 6. Bonnie (France), 7. Bull (Canada). Cultivar Targovishte 1 (St) was used for comparison.

The agrotechnical management over the growing season was carried out in accordance with the biophysiological and technological requirements of the bird's foot trefoil.

The mode of soil tillage was reported in another publication (Churkova, 2012). Sowing was conducted by hand, broadcast at the sowing rate of 0.012 t/ha.

Fertilizing with phosphorus and potassium was conducted as reserve application at the dose of 0.16 t/ha active ingredient together with the basic soil tillage, and nitrogen was applied once before sowing at the dose of 0.06 t/ha active ingredient.

The bird's-foot-trefoil for forage harvesting was conducted at the stage of budding-early flowering.

Chemical composition of dry mass yield of bird's foot trefoil cultivars

The samples for the chemical analysis of forage were taken in bud-formation period – the beginning of flowering of each undergrowth during the years of the experimental period. Plant materials were left to dry under natural conditions. Immediately prior milling, the samples had been dried in laboratory drying-furnace at a temperature of 60^0C in order to facilitate the milling. The milling was done in a laboratory mill with a size of particles 1.0 mm. The following indicators were determined from the absolutely dry samples: crude protein, crude fiber, crude ash, nitrogen-free extractable substances, crude fat, calcium and phosphorus.

The crude protein values were reported on the base of nitrogen, which was obtained through conversion of N x 6.25 after the Kjeldahl method (AOAC, 1995). Crude fibers were determined after Weende method on the basis of solubility of cellulosic components in solutions of sulphuric acid and potassium hydroxide. Crude ash content was determined by burning in a muffle furnace at t^0 550^0C. Crude fats were extracted in Soxhlet extractor with ordinary ether. Nitrogen-free extractable substances were calculated on the basis of the obtained crude protein, crude fat, crude fiber, mineral substances.

Statistics analyses in bird's foot trefoil cultivars and populations

The statistical data processing was carried by variation-statistical method (Lidanski, 1988), which included: average value (x), minimal (min) and maximal (max) values. The degree of variability was expressed by variation coefficient (CV%) according to the scheme of Mamaev: up to 7% – very low; 7.1-12% – low; 12.1-20% – average; 20.1%-40% – high and over 40% – very high. The initial data were processed according to the method of dispersion analysis. Correlation dependences between main indicators of the chemical composition and dry matter yield, the height of plants and proportion of stems, leaves and generative organs were calculated by correlation coefficient of Brave and Pearson (r) by Microsoft Excel.

Results and Discussion

Content of crude protein

Data on crude protein content by years and average for the period for individual cultivars and populations of bird's-foot-trefoil are presented in Table 1. In the first experimental year, crude protein varies slightly, from 156.7 g kg^{-1} DM to 199.3 g kg^{-1} DM, at an average value of X = 170.67 g kg^{-1} DM. High values of crude protein were also reported in the Targovishte 1 with 183.0 g kg^{-1} DM. The lowest protein content was registered in the biomass of Bonnie cultivar, and the

highest one in Frilo. The excess of this quality indicator in Frilo compared to the standard was respectively 16.3 g kg^{-1} DM. The difference between the maximum and minimum value of crude protein in different cultivars was 42.6 points. The established lower levels of the indicator for the other cultivars are due to the lower share of bird's-foot-trefoil in the grassland and respectively in the cut fodder mass.

In the second year (2017), the amount of crude protein ranged from 136.8 g kg^{-1} DM to 197.9 g kg^{-1} DM. The highest value for this indicator was reported in the feed of variety Targovishte 1 (197.9 g.kg^{-1} CB). Bull cultivar (197.5 g kg^{-1} DM) had a similar value of crude protein. The difference between the value of crude protein in the standard cultivar and that of Bull cultivar was 61.1g.kg^{-1} DM, respectively. Both cultivars (Targovishte 1 and Bull) have good values in terms of crude protein, which makes them suitable for growing in soil and climatic conditions of the region. With a coefficient of variation CV = 11.82%, the degree of variability in this indicator is low. The established high content of crude protein in the second year corresponds to the highest productivity of the tested cultivars under the same conditions (Churkova, 2012). The established lower levels of the indicator for the other cultivars are due to the lower share of bird's-foot-trefoil in the grassland and respectively in the cut fodder mass.

In the third experimental year, the crude protein had the highest percentage share in the fodder mass of Polom – 187.3 g kg^{-1} DM. A minimum amount of crude protein was found in Bonnie cultivar with 153.0 g kg^{-1} DM. Alvena (184.6 g kg^{-1} DM) and Bull (184.8 g kg^{-1} DM) had almost similar values in this indicator. A low degree of variability was calculated according to the value of the variation coefficients (CV = 7.88) at an average value of 172.66 g kg^{-1} DM, respectively.

In the fourth year, the crude protein ranged from 169.9 g kg^{-1} DM to 187.8 g kg^{-1} DM. The lowest protein content was registered in the biomass of Alvena cultivar, and the highest one in Lotanova. Polom (184.7 g kg^{-1} DM), Bull (183.8 g kg^{-1} DM) and the standard cultivars Targovishte 1 (183.5 g kg^{-1} DM) showed identical values on this indicator. The degree of variability is very low (CV = 4.00), and the average value of crude protein content in all crude protein is 179.37 g kg^{-1} DM.

The averaged data show that Bull cultivar, which slightly exceeded the standard, is of interest in terms of crude protein content. All other cultivars have crude protein lower than the standard. Its values varied from 155.1 to 181.1 g kg^{-1} DM, with an average value of 174.69 g kg^{-1} DM, low degree of variation (CV = 5.31) and low degree of standard deviation (SD – 9.28).

The crude protein content in bird's foot trefoil corresponds to that reported by Ramirez-Restrepo et al. (2006), who found that the variation was from 10.56 to 21.93% and decreased with increasing maturity. This result is also in agreement with the findings of Karabulut et al. (2006), stating that the level of protein in the flowering phase is 17.15%. The content of crude protein in hay is closely related to the stages of maturity, with a tendency to decrease with age of the plants (Kamalak et al., 2005). The low protein is due to a protein reduction in the leaves and stems, as the stems with their lower protein concentration make up more of the grasses in more mature fodders (Buxton, 1996).

Crude fat content

Crude fats are the most concentrated source of energy for a living organism, improve the taste of feed, facilitate the dissolution and absorption of fat-soluble vitamins A, D, E and K. Their content in the dry matter in 2016 (Table 2) varies from 24.9 g kg^{-1} DM (Targovishte 1) to 42.3 g kg^{-1} DM (Alvena). In contrast to crude protein in crude fats, we observe a significant

Table 1. Content of crude protein (g kg^{-1} DM) of bird's foot trefoil cultivars over the years and average for the period 2016-2019

Cultivars	2016	2017	2018	2019	Average for the period
Targovishte 1	183.0	197.9	158.2	183.5	180.7
Alvena	180.0	181.8	184.6	169.9	179.1
Lotanova	156.7	172.8	170.4	187.8	171.9
Frilo	199.3	177.9	170.3	172.5	180.0
Polom	160.2	167.5	187.3	184.7	174.9
Bonnie	157.1	136.8	153.0	173.4	155.1
Bull	158.4	197.5	184.8	183.8	181.1
X	170.67	176.03	172.66	179.37	174.69
SD	16.82	20.81	13.60	7.17	9.28
VC	9.86	11.82	7.88	4.00	5.31
Min	156.7	136.8	153.0	169.9	155.1
Max	199.3	197.9	187.3	187.8	181.1

difference in this indicator in different cultivars, which is evident from the value of the variation coefficient (CV = 16.63). According to him, the degree of variability is medium, and that of the standard deviation is low (SD – 5.67).

In the second experimental year, their dry matter content varied from 27.8 g kg^{-1} DM (Bonnie cultivar) to 47.1 g kg^{-1} DM (Targovishte 1). All tested cultivars had a lower fat content than the standard cultivar. They have an average degree of variability according to the value of the variation coefficient, which is on the border with high variability (CV = 19.43).

The content of crude fat in the dry matter in the third year (2018) of the development of grassland varied from 29.0 g kg^{-1} DM (Lotanova) to 42.7 g kg^{-1} DM (Frilo). It is noteworthy that crude fats were in greater quantities in the second and third years than in the first and fourth years of the experiment. The degree of variation in this indicator is also (CV = 20.67) the highest compared to the other years of the study.

In 2020, the opposite trend was observed in terms of the fat amount, which is expressed in the fact that in Targovishte 1 it had a minimum value (26.6 g kg^{-1} DM), in contrast to the previous two years. A maximum value of 38.3 g kg^{-1} DM was reported for Frilo, and similar values of crude fat were recorded for Bonnie 31.4 g kg^{-1} DM and Bull 31.4 g kg^{-1} DM. The content of the studied indicator varied to a greater extent, which determined its variability degree as average (CV = 12.58%).

Crude fats on average for the study period had a slight variation degree in the tested cultivars (CV = 8.83) and a low relative share of dry matter composition. Their values ranged from 31.0 to 39.8 g kg^{-1} DM, with the highest content in Frilo. The content of crude fat in the tested cultivars both by years and on average for the period varied within narrow limits, which confirmed the results obtained by Putri et al. (2019) for insignificant variation in this indicator for legumes, respectively from 2.29 to 4.15%.

Crude fibers content

Significant differences were observed in the crude fiber (Table 3) during the years of the experimental period. The highest content of crude fiber was registered in Lotanova (337.2 g.kg^{-1} DM) in the first year, while the lowest one in Alvena (271.7 g.kg^{-1} DM). The difference in the values of crude fiber in the cultivars is small, which is evident from the very low variability degree in this indicator (VC = 7.93). Compared to the first year, the amount of crude fiber in all cultivars was significantly lower. Bonnie cultivar recorded a value of 195.6g.kg^{-1} DM for crude fiber, and the average value was 252.3 g.kg^{-1} DM. In the third year, the values were close to those in the first year and were higher than the crude fiber in the second year. The highest value was registered in Frilo (340.2 g.kg^{-1} DM), and the lowest in Bull (340.2 g.kg^{-1} DM). In the fourth year, the highest crude fiber values were registered in all cultivars, with an average value of 338.43 g.kg^{-1} DM, and there was an average degree of variability according to the variation coefficients VC = 11.18. On average for the study period, the standard cultivar had the most amount of fiber (315.1 g.kg^{-1} DM) and Bonnie cultivar had the least amount (269.5 g.kg^{-1} DM), with a very low degree of variability VC = 5.30 and the lowest value of the standard deviation (SD = 15.93).

Ganskopp and Bohnert (2001) reported statistically significant differences in the fiber content of plants during their different growth stages. The lower fiber content during the initial development of plants and their gradual increase at a later stage is due to a decrease in the leaf/stem ratio as plant growth progresses, as well as an increase in the rate of stem biomass (Tan et al., 2003). Higher fiber content in grass species during the growth stages in the ripening period has also been reported by other researchers (Elgersma et al., 2013; Elgersma & Soegaard, 2016).

Table 2. Content of crude fat (g kg^{-1} DM) of bird's-foot-trefoil cultivars over the years and average for the period 2016-2019

Cultivars	2016	2017	2018	2019	Average for the period
Targovishte 1	24.9	47.1	50.6	26.6	37.3
Alvena	42.3	44.0	34.1	29.8	37.6
Lotanova	29.6	37.0	29.0	28.5	31.0
Frilo	38.3	40.0	42.7	38.3	39.8
Polom	33.2	34.3	37.3	35.1	35.0
Bonnie	35.2	27.8	47.2	32.4	35.7
Bull	35.2	29.3	32.1	31.4	32.0
X	34.10	37.07	39.00	31.73	35.49
SD	5.67	7.20	8.06	3.99	3.13
VC	16.63	19.43	20.67	12.58	8.83
Min	24.9	27.8	29.0	26.6	31.0
Max	42.3	47.1	50.6	38.3	39.8

Crude ash content

The content of crude ash (Table 4) in the different cultivars in the first year varied from 71.9 g.kg^{-1} DM to 87.0 g.kg^{-1} DM, with an average value of X = 81.49. The content of mineral substances in dry matter in Lotanova and Frilo is the same (86.4 g kg^{-1} DM) and similar to that of Bonnie (87.0 g kg^{-1} DM). The values of crude ash in the dry mass of Polom and Bull were almost identical, respectively 79.1 and 77.9 g kg^{-1} DM.

Crude ash in the second experimental year was affected by cultivars to the highest degree compared to the amount in other years. This is evident from the values of the variation coefficient VC = 11.88, according to which the degree of variability in this indicator is average. The limits of variation were from 65.4 (Targovishte 1) to 97.7 g.kg^{-1} DM (Bonnie) with an average value of 84.33 g.kg^{-1} DM. The difference in the values of crude ash in the fodder mass of Alvena and Polom was insignificant, respectively 83.2 and 84.8 g kg^{-1} DM. Due to the fact that in bulky fodder, fats are present with a small relative share of dry matter composition, ash is not involved in determining the digestibility and nutritional value.

In the third year of the experimental period (2018), the maximum value of minerals in dry matter was reported for Alvena (77.5 g kg^{-1} DM) and the minimum for Bull (67.9 g kg^{-1} DM), with an average value of X = 73.10 and the lowest degree of variability according to the value of the variation coefficient VC = 4.16. With the exception of Bull cultivar, the values of crude ash in the fodder mass of the other cultivars were almost similar, 71.7 g kg^{-1} DM and 77.5 g kg^{-1} DM, respectively.

In the last year of the experiment (2019) the largest amount of crude ash was registered in dry matter of Bull (72.7 g kg^{-1} DM), while 'Targovishte 1' and Alvena had the least (62.2 and 62.6 g kg^{-1} DM). The values of this indicator in theodder mass

Table 3. Content of crude fibers (g kg^{-1} DM) of bird's-foot-trefoil cultivars over the years and average for the period 2016-2019

Cultivars	2016	2017	2018	2019	Average for the period
Targovishte 1	326.0	265.5	335.6	333.4	315.1
Alvena	271.7	247.6	308.0	387.4	303.7
Lotanova	337.2	288.8	304.9	308.7	309.9
Frilo	305.6	255.6	340.2	352.9	313.6
Polom	292.7	290.7	305.2	312.7	300.3
Bonnie	287.4	195.6	304.9	290.0	269.5
Bull	282.5	222.5	277.9	383.9	291.7
X	300.4	252.3	310.9	338.4	300.5
SD	23.84	34.4	21.10	37.84	15.93
VC	7.93	13.65	6.78	11.18	5.30
Min	271.7	195.6	277.9	290.0	269.5
Max	337.2	290.7	340.2	387.4	315.1

Table 4. Content of crude ash (g kg^{-1} DM) of bird's-foot-trefoil cultivars over the years and average for the period 2016-2019

Cultivars	2016	2017	2018	2019	Average for the period
Targovishte 1	71.9	65.4	75.6	62.2	68.8
Alvena	81.7	83.2	77.5	62.6	76.3
Lotanova	86.4	91.1	71.7	67.8	79.3
Frilo	86.4	81.1	72.9	64.1	76.1
Polom	79.1	84.8	72.4	64.4	75.2
Bonnie	87.0	97.7	73.7	68.1	81.6
Bull	77.9	87.0	67.9	72.7	76.4
X	81.49	84.33	73.10	65.99	76.24
SD	5.62	10.02	3.04	3.76	3.97
VC	6.89	11.88	4.16	5.69	5.21
Min	71.9	65.4	67.9	62.2	68.8
Max	87.0	97.7	77.5	72.7	81.6

Table 5. Correlation dependences among the yield, percentage share of bird's foot trefoil in the grassland and qualitative indicators

Indicators	Dry matter yield, kg ha^{-1}	Crude protein, g kg^{-1} DM	Crude fat, g kg^{-1} DM	Crude fiber, g kg^{-1} DM	Crude ash	Height of stems of bird's foot trefoil, cm	% bird's-foot-trefoil
Dry matter yield, kg ha^{-1}	1						
Crude protein, g kg^{-1} DM	-0.2232	1					
Crude fats , g kg^{-1} DM	-0.2663	0.1663	1				
Crude fibers, g kg^{-1} DM	0.0558	0.7851	0.2395	1			
Crude ash, g kg^{-1} DM	0.5347	-0.7146	-0.3564	-0.6560	1		
Height of stems of bird's foot trefoil, cm	-0.2477	-0.2924	0.1071	-0.3191	-0.2699	1	
% bird's foot trefoil	0.2681	0.8516	0.0741	0.3015	-0.4012	0.1728	1

of 'Frilo and Polom' are similar, respectively 64.1 and 64.4 g kg^{-1} DM.

On average for the study period, the highest value of crude ash was registered for Bonnie (81.6 g kg^{-1} DM) and the lowest for the standard variety (68.8 g kg^{-1} DM). Both values determine the very low degree of variability in this indicator, according to the coefficient of variation (CV = 5.21) and the low value of the standard deviation.

According to Putri et al. (2019) the amount of ash varies from 6.82% to 10.33%, but no significant difference is observed among the feed. The high average mineral content in legumes, with statistically significant differences ($P < .05$) is explained by the higher mineral content in the leaves than in the stems (Myrvang et al., 2016) and the higher stem/leaf ratio of other botanical groups (Cherney et al., 1990). In general, higher mineral content in legumes and other plants compared to grasses has been reported by other researchers (Lindström et al., 2014). Differences in mineral content between different plant species, between the same plant species under different conditions and between the same plant species at different growth stages have also been reported by other researchers (Lindstrom et al., 2014; Elgersma & Soegaard, 2016; Schlegel et al., 2016).

The established strong positive correlation (Table 5) between the content of crude protein and the percentage share of bird's-foot-trefoil in the grassland (r = 0.8516) on the one hand, and the content of crude fiber and crude protein (r = 7851) on the other hand, showed the interdependence among the indicators. Crude ash and dry matter yield are also positively correlated (r = 5347). The degree and direction of the connection between these indicators is important because they make it possible to predict and accelerate the selection processes in the direction of fodder quality. The lack of significant, divergent and proven values in most cases for the correlation coefficients of dry matter yield and the content of crude proteins, crude fat, stem height (r = -0.2232; -0.2663; -0.2477) shows that the dependence is weak and the signs are independent. Crude ash is negatively related to the content of crude protein (r = -0.7146), crude fat (r = -0.3564), crude fiber (r = -0.6560). This shows that these traits are genetically independent and the selection in terms of quality indicators would not negatively affect productivity.

Conclusions

The chemical analysis on fodder of seven cultivars of bird's foot trefoil with different origin in mountain conditions presented Bull cultivar as the most promising in terms of the amount of crude protein, while Bonnie cultivar was most productive in terms of crude fiber and minerals. Fats registered maximum values for Frilo cultivar. The indicators of crude protein, crude fiber and crude ash showed a very low degree of variability, while for the indicator of fat it was low.

The established strong positive correlation between the content of crude protein and the percentage share of bird's foot trefoil in grassland (r = 0.8516) on the one hand, and the content of crude fiber and crude protein (r = 7851) on the other hand, showed the interdependence between the indicators. This determines the benefits of growing these cultivars in mountain conditions and their significance for animal feeding.

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