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Influence of treatment with herbicides on the basic chemical composition on bird's foot trefoil (*Lotus corniculatus* L.) swards

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Abstract: During the period 2020-2022 in the experimental field of Research Institute of Mountain Stockbreeding and Agriculture – Troyan, a research experiment with bird's foot trefoil (*Lotus corniculatus* L.), cultivar Targovishte 1 was conducted to determine the impact of herbicide treatment on the basic chemical composition of the forage mass. The experiment was set up using the block method with the following variants: 1. Control- untreated (C); 2. Kalam at a dose of 20 g/da; 3. Kalam at a dose of 40 g/da 4. Focus ultra at a dose of 100 ml/da; 5. Focus ultra at a dose of 200 ml/da. Focus ultra herbicide (active substance cycloxydim 100 g/l) at the dose of 100 ml/da was found to exhibit positive effect on crude protein content, quantity of crude fats and mineral substances. The herbicide Kalam (dicamba 600 g/kg and tritosulfuron 125 g/kg) at a dose of 20 g/da stimulated the amount of the macroelement calcium to the highest extent, while its higher dose increased the phosphorus content in the biomass of bird's foot trefoil. The two herbicides did not affect the amount of nitrogen-free extractable substances (NFE). The favourable combination of high protein and low crude fiber determines the herbicide Focus Ultra at a dose of 100 ml/da as suitable for application in the practice.

Keywords: bird's foot trefoil; herbicides; basic chemical composition

INTRODUCTION

Bird's foot trefoil (*Lotus corniculatus* L.) is a perennial legume with good adaptability to poorly fertile soils (Moye, 2018) with acid reaction (Churkova & Bozhanska, 2016). Due to these advantages combined with its high forage quality (Christensen et al., 2015; Vasileva & Naydenova, 2018; Churkova & Churkova, 2022; Greenland, 2022), excellent resistance to grazing (Barry et al., 2003), ability to self-seed and durability define it as a suitable component for establishing artificial meadows and pastures (Owens et al., 2012; Vasileva et al., 2019).

A significant disadvantage of bird's foot trefoil is its slow growth rate during the early stages of its development due to high competition from weed species (Churkova, 2013; Mashece et al., 2024). In the absence of control, they not only reduce crop yield but also forage quality (Windle et al., 2014). Weed infestation in grasslands during this period necessitates the search for appropriate methods to control undesirable species.

Usually such a method is the chemical one. Therefore, the application of herbicides and their efficacy, is critical to obtain sustainable productivity from forage grasslands (Atis et al., 2012). Several authors (Jansen & Gregorio, 2004; Rosegrant et al., 2009), define herbicide efficacy and crop safety as complex and include plant species, plant size, growth phase, soil composition and physical properties, soil moisture, temperature and relative humidity. Very few herbicides have been registered for weed control in bird's foot trefoil grassland. Herbicides certified for weed control include 2.4-D, MCPP and dicamba as active ingredients. The herbicide glyphosate essentially interferes with plant photosynthesis, hindering nutrient and water uptake, thereby impairing their growth and development (Hood et al., 2013).

The digestibility of protein in the dry matter of bird's foot trefoil fodder and the fibre content depends on the work to improve the conditions under which the plants are grown (Grabber et al., 2014; 2015). Properly conducted weed control ensuring reduced density of undesirable species in the stand is important for improving forage quality.

The aim of this study is to determine the effect of herbicide treatments on the basic chemical composition on forage mass of bird's-foot trefoil.

MATERIAL AND METHODS

The study was conducted in the period 2020-2022 in the experimental field of Research Institute of Mountain Stockbreeding and Agriculture-Troyan on light grey pseudopodzolic soil under non-flooded conditions with bird's foot trefoil (*Lotus corniculatus* L.), cultivar Targovishte 1. The experiment was laid out using the block method in 4 replications with a plot size of 5 m^2 with the following treatments: 1. Control- untreated (C); 2. Kalam (dicamba 600 g/kg and tritosulfuron 125 g/kg) - 20 g/da; 3. Kalam (dicamba 600 g/kg and tritosulfuron 125 g/kg) - 40 g/da; 4. Focus ultra - 100 ml/da (active substance cycloxydim 100 g/l); 5. Focus ultra - 200 ml/da (active substance cycloxydim 100 g/l). The sowing was carried out in spring at a seeding rate of 1.2 kg/da at a row spacing of 12.5 cm, and the area was railed before and after. We applied the herbicides at 2-4 leaf stage of bird's foot trefoil with a backpack sprayer at a working solution rate of 50 l/da.

In the phenophase budding-bloom initiation, one subplot was harvested in the first year and by two each in the remaining years of the experimental period. Average samples weighing 500 g were taken from each variant of the research experiment and then dried to a constant dry weight. Chemical composition was presented by variants of plant samples from the total green mass of the grassplots nnually by subgrowth and averaged by year and over the study period in g. kg^{-1} DM, and the following chemical parameters were determined: Crude protein (CP) - by Kjeldahl method using the formula CP = N x 6.25 (Sandev, 1979); Crude fibre (CF) - by treatment with solutions of 1.25% (w/v) H_2SO_4 and 1.25% (w/v) NaOH according to the Weende analysis (AOAC, 2007); Ash - degradation of organic matter by gradual combustion of the sample in a muffle furnace at 550°C; calcium (Ca) by Stotz; phosphorus (P) by Guericke and Kurmis; crude fat (CF) - by extraction in a *Soxhlet*-type extractor with non-polarized organic solvent, nitrogen-free extractable substances (NFE) = 100 - (CP, % + CF, % + CF, % + Ash, % + Humidity, %), converted to g kg^{-1}.

Statistical analysis was performed on the obtained data using the software products *Analysis Toolpak for Microsoft Excel 2010* and *Statgraphics Plus v.2.1.* Average values (x), standard deviation (SD), coefficient of variation (VC), minimum (Min) and maximum values (Max) were calculated. The statistical data processing was carried out by variation statistical method (Lidanski, 1988), which included: mean value (x), minimum (min) and maximum (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 variance analysis.

RESULTS AND DISCUSSION

The basic chemical composition of the above-ground plant biomass samples of bird's foot trefoil for 2020 after treatment with Kalam and Focus Ultra herbicides is presented in Table 1. The results obtained from the biochemical analysis showed increased crude protein (201.1 g kg^{-1} DM), crude fat (40.9 g kg^{-1} DM) and calcium (36.8 g kg^{-1} DM) contents in the bird's foot trefoil after treatment with Kalam herbicide at a dose of 20 g/da. Average crude protein value of all treatments X = 188.62 g kg^{-1} DM, the remaining treatments had crude protein values ranging only slightly from 183.0 to 189.8 g kg^{-1} DM, respectively. The high values of crude protein are explained by the favorable influence of herbicides, which reduce the harmful effect of weeds and increase the relative proportion of bird's foot trefoil in the grassland. This is also proved by the extremely low values of coefficient of variation (VC = 3.97%) and standard deviation SD = 7.48 of crude fats.

The dose of Kalam herbicide influenced the crude fat content, which is evident from their lowest value when treated at a dose of 40 g/da (28.0 g kg^{-1} DM) and the highest when the same herbicide was applied at a dose of 20 g/da (40.9 g kg^{-1} DM) with a mean value of 35.16 g kg^{-1} DM and an average degree of variability according to the coefficient of variation (VC = 14.39%). The tendency is impressive for a slight increase in crude fibre relative to the control. The herbicide treatment significantly increased the amount of crude fibre, which was 348.1 g kg^{-1} DM after the imported herbicide Focus Ultra at a dose of 200 ml/da, with an excess of 70.5 g kg^{-1} DM over the untreated variant. The degree of variability according to the coefficient of variation (VC = 8.69%) was low and the standard deviation was SD = 8.69. The variation in mineral content was also low (VC = 8.04%) and was highest in the Focus Ultra treatment at a dose of 100 ml/da, with insignificant excess over the control. The macroelement calcium had the highest variability of all parameters in the first year according to the value of the coefficient of variation (VC = 23.98%). A decreasing trend of NFE content with herbicide application was found for weed control, respectively, at 330.0 g kg^{-1} DM the control showed a decrease of 272.6 g kg^{-1} DM with mean value X = 304.68 g kg^{-1} DM and low variability according to coefficient of variation (VC = 7.62%). Phosphorus varied from 1.49 to 2.41 g kg^{-1} DM with maximum content in forage mass after applied herbicide Focus Ultra at both doses.

In the second experimental year, the crude protein content (Table 2) ranged from 128.8 to 153.5 g kg^{-1} DM, crude fat from 18.6 to 25.9 g kg^{-1} DM, crude fibre from 366.1 to 525.9 g kg^{-1} DM, crude ash from 56.7 to 138.4 g kg^{-1} DM, and nitrogen-free extractable substances from 131.50 to 304.4 g kg^{-1} DM. The differences in calcium content by variants in the above-ground biomass of bird's foot trefoil varied relatively slightly, from 10.0 to 21.0 g kg^{-1} DM, and of phosphorus from 1.1 to 1.8 g kg^{-1} DM, respectively. The forage mass of the grassland treated with the herbicide Focus Ultra at a rate of 100 ml/da realized the highest contents of crude protein (153.5 g kg^{-1} DM), crude ash (138.4 g kg^{-1} DM) and calcium (21.0 g kg^{-1} DM) and had a favorable chemical composition

Table 1. Main chemical content (g kg^{-1} DM) of forage mass of bird's foot trefoil treated with herbicides for 2020

Variants	Crude protein	Crude fats	Crude fiber	Ash	NFE	Calcium	Phosphorus
1. Control - untreated (C)	185.8	35.4	277.6	73.4	330.0	21.3	1.93
2. Kalam 20 g/da	201.1	40.9	293.9	69.2	296.0	36.8	1.49
3. Kalam 40 g/da	183.4	28.0	303.5	62.3	324.5	25.7	2.17
4. Focus Ultra - 100 ml/da	189.8	38.7	297.2	76.7	300.3	27.9	2.41
5. Focus Ultra - 200 ml/da	183.0	32.8	348.1	66.9	272.6	21.3	2.41
X	188.62	35.16	304.06	69.70	304.68	26.60	2.08
SD	7.48	5.06	26.41	5.60	23.22	6.38	0.39
VC	3.97	14.39	8.69	8.04	7.62	23.98	18.55
Min	183.00	28.00	277.60	62.30	272.60	21.30	1.49
Max	201.10	40.90	348.10	76.70	330.00	36.80	2.41

in relation to forage quality. The higher dose of the same herbicide (Focus Ultra - 200 ml/da) had a favorable effect on forage quality due to the reduced crude fibre content (366.1 g kg^{-1} DM) with an average value of X = 426.80 g kg^{-1} DM. According to the coefficient of variation, the degree of variability in this parameter (VC = 23.98%) is average and the value of standard deviation is SD = 60.79.

The coefficient of variation is also the lowest (VC = 6.26%), which is explained by the non-significant difference between the minimum and maximum value of this indicator. Minerals had the highest coefficient of variation (VC = 41.74%), which determines the very high degree of variability due to its high value when treated with the herbicide Focus Ultra at a dose of 200 ml/da. The non-significant difference in the values of the indicators in the basic chemical composition is explained by the significantly lower values of temperature and precipitation, which determine the available dry and hot summer months (July and August), combined with lower relative humidity compared to the first and third experimental years. The data obtained are related to the effect of herbicides on the morphological composition of the grasslands, which showed a higher relative proportion of leaves in the forage mass of bird's foot trefoil.

In the third year of the study period, the crude protein content (Table 3) exceeded that obtained in the second experimental year and was similar in values to that obtained in the first year. The insignificant difference in crude protein by variants is remarkable. This indicates that the applied types of herbicides and their doses do not affect this indicator, which is evident from the value of the coefficient of variation (VC = 2.22%). A difference of 10.67 points between the minimum and maximum values determines the very low variability in this indicator. Treatment of bird's foot trefoil with the herbicide Kalam at a dose of 20 g/da realized the highest protein (160.4 g kg^{-1} DM) and calcium (22.4 g kg^{-1} DM) and the lowest crude fiber content (306.0 g kg^{-1} DM). There was a significant excess in the amount of nitrogen-free extractable substances with increasing age of grassland. This is evident from their mean value per year: for the first year 304.68, for the second year 231.80 and for the third year 422.34 g kg^{-1} DM and mean degree of variability according to the coefficient of variation (VC = 12.92%).

The crude fat content only exceeded the untreated control in the Focus Ultra variant at a dose of 100 ml/da (36.0 g kg^{-1} DM), with a high degree of variability according to the coefficient of variation (VC = 36.43%). Except for the variant treated with the herbicide Kalam at a dose of 20 g/da,

Table 2. Main chemical content (g kg^{-1} DM) of forage mass of bird's foot trefoil treated with herbicides for 2021

Variants	Crude protein	Crude fats	Crude fiber	Ash	NFE	Calcium	Phosphorus
1.Control - untreated (C)	128.8	23.7	432.0	58.1	259.1	14.4	1.1
2. Kalam 20 g/da	139.1	20.5	392.8	56.7	292.4	12.2	1.3
3. Kalam 40 g/da	141.6	25.9	525.9	77.8	131.5	10.0	1.8
4. Focus Ultra - 100 ml/da	153.5	18.6	417.2	138.4	171.6	21.0	1.5
5. Focus Ultra - 200 ml/da	139.6	21.5	366.1	71.3	304.4	14.4	1.4
X	140.52	22.04	426.80	80.46	231.80	14.40	1.42
SD	8.80	2.84	60.79	33.59	76.44	4.12	0.26
VC	6.26	12.86	14.24	41.74	32.98	28.58	18.23
Min	128.80	18.60	366.10	56.70	131.50	10.00	1.10
Max	153.50	25.90	525.90	138.40	304.40	21.00	1.80

calcium was half the amount (22.4 g kg^{-1} DM) of the control. The phosphorus content ranged from 11.5 to 12.2 g kg^{-1} DM, which determined the very low variability in this parameter (VC = 2.32%) and the mean value X = 11.98 g kg^{-1} DM.

On average over the study period (Table 4), the crude protein content of the bird's foot trefoil biomass was not affected by the herbicide treatments and the values of the treatments for this trait exceeded the control insignificant. This is evident from the minimum and maximum values which were 156.20 and 166.87 g kg^{-1} DM, respectively. The variation in this indicator was the lowest (VC = 2.74%) compared to the other indicators of the basic chemical composition. Herbicide-treated grasslands exceeded the untreated control in crude protein content, with the maximum protein value after the application of Focus Ultra at 100 ml/da (165.7 g kg^{-1} DM). The crude fat content of bird's foot trefoil biomass was not significantly

Table 3. Main chemical content (g kg^{-1} DM) of forage mass of bird's foot trefoil treated with herbicides for 2022

Variants	Crude protein	Crude fats	Crude fiber	Ash	NFE	Calcium	Phosphorus
1. Control - untreated (C)	154.0	21.8	311.8	67.6	444.8	21.3	12.1
2. Kalam 20 g/da	160.4	19.0	306.0	54.4	439.4	22.4	12.0
3. Kalam 40 g/da	153.6	15.3	326.8	53.3	471.8	14.6	12.2
4. Focus Ultra - 100 ml/da	153.9	36.0	431.6	49.2	329.3	12.4	11.5
5. Focus Ultra - 200 ml/da	159.9	18.7	340.9	54.1	426.4	12.4	12.1
X	156.36	22.16	343.42	55.72	422.34	16.62	11.98
SD	3.47	8.07	51.13	6.96	54.58	4.87	0.28
VC	2.22	36.43	14.89	12.49	12.92	29.32	2.32
Min	153.60	15.30	306.00	49.20	329.30	12.40	11.50
Max	160.40	36.00	431.60	67.60	471.80	22.40	12.20

Table 4. Main chemical content (g kg^{-1} DM) of forage mass of bird's foot trefoil treated with herbicides averaged over the period

Variants	Crude protein	Crude fats	Crude fiber	Ash	NFE	Calcium	Phosphorus
1. Control - untreated (C)	156.2	27.0	340.5	66.4	344.6	19.0	5.0
2. Kalam 20 g/da	166.9	26.8	337.8	60.1	342.6	23.8	4.9
3. Kalam 40 g/da	159.5	23.1	378.5	64.5	309.3	16.8	5.4
4. Focus Ultra - 100 ml/da	165.7	31.1	382.0	88.1	267.1	20.4	5.1
5. Focus Ultra - 200 ml/da	160.8	24.3	351.7	64.1	334.5	16.0	5.3
X	161.83	26.45	358.09	68.63	319.61	19.21	5.16
SD	4.43	3.08	20.91	11.12	32.57	3.11	0.19
VC	2.74	11.64	5.84	16.21	10.19	16.19	3.63
Min	156.20	23.07	337.83	60.10	267.07	16.03	4.93
Max	166.87	31.10	382.00	88.10	344.63	23.80	5.39

affected by the applied herbicides and the values of the treated variants for this trait did not exceed the control. Only the treatment of bird's foot trefoil with the herbicide Focus Ultra at a dose of 100 ml/da showed an increase in crude fat content compared to the control (31.1 g kg^{-1} DM). Except for the grassland treated with the herbicide Focus Ultra at a dose of 100 ml/da (337.8 g kg^{-1} DM), all other grasslands showed an increasing trend in crude fiber content compared to the control. The variation in this parameter ranged from 337.83 to 382.0 g kg^{-1} DM and determines the very low variability in this indicator according to the value of the coefficient of variation (VC = 5.84%) with mean value X = 351.7 g kg^{-1} DM.

The average value of the ash indicator was 64.1 g kg^{-1} DM, with a slight excess over the control (21.7 g kg^{-1} DM) observed in the variant treated with the herbicide Focus Ultra at a dose of 100 ml/da. Nitrogen-free extract substances, calcium and phosphorus are essential for determining the taste qualities of forage. The carbohydrate content (NFE) in dry matter of bird's foot trefoil ranged from 267.07 to 344.63 g kg^{-1} DM. There was a decreasing trend in the amount of NFE in the treatments, and a similar but lower value than the control was recorded in the treatment with the herbicide Kalam at a dose of 20 g/da (342.6 g kg^{-1} DM). The variation in this parameter was low (VC = 10.19%) and the mean value X = 334.5 g kg^{-1} DM. The bird's foot trefoil after the imported herbicide Kalam at a dose of 20 g/da increased the amount of calcium, with an excess over the control of 4.8 g kg^{-1} DM, respectively. Higher values of the macro element were also observed after treating the plants with the herbicide Focus Ultra at a dose of 100 ml/da. The average value for the study period was 19.21 g kg^{-1} DM and was close to that of the control (19.0 g kg^{-1} DM). There was a slight increase in phosphorus content in herbicide-treated grasslands in treatments 3, 4 and 5, but with a marginal non-significant difference to the control. The variation of the parameter was from 4.93 to 5.39 g kg^{-1} DM with mean value X = 5.16 g kg^{-1} DM.

Comparing the results by years, a trend of decreasing protein with increasing plant maturity was found, confirming the results obtained by Karabulut et al. (2006). The decrease of CP with the advancing age of herbage was due to decrease in protein in leaves and stems. In the later stages of plant development, stems are a larger fraction of plant mass, have a lower protein content, which determines the reduced protein content in forage (Buxton, 1996). The lower crude fiber content during initial plant development corresponds with studies done by Elgersma et al. (2013) and Elgersma & Soegaard (2018). For most of the parameters studied, the difference between the maximum mean values of the applied herbicides with the control was different, indicating that the action of herbicides depends on their type, the composition of the active substance and the physiology of the plant species on which they are applied.

The values of the chemical composition indicators in the bird's foot trefoil forage are due to changes in the metabolism of the species originating from herbicide spraying, which activates the secondary metabolism of the plant (Agostinetto et al., 2020). This induces defense and overcoming stress and stimulates their growth and development (Metlen et al., 2009). The action of the applied herbicides and their influence on the basic chemical composition of the forage mass is due to the demonstrated low phytotoxicity to plants and the high selectivity of herbicides.

CONCLUSIONS

Of the herbicides studied in the grassland from bird's foot trefoil, the effect of the herbicide Focus Ultra at a dose of 100 ml/da had a positive effect on crude protein content, crude fat content and mineral content.

Treatment with the herbicide Kalam at a dose of 20 g/da stimulated the amount of the macronutrient calcium to the highest extent and reduced the fiber content of dry matter, while its higher dose increased the phosphorus content of the bird's foot trefoil biomass. The amount of NFE, was not affected by the action of the tested herbicides, which is evident from its highest value in the control.

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